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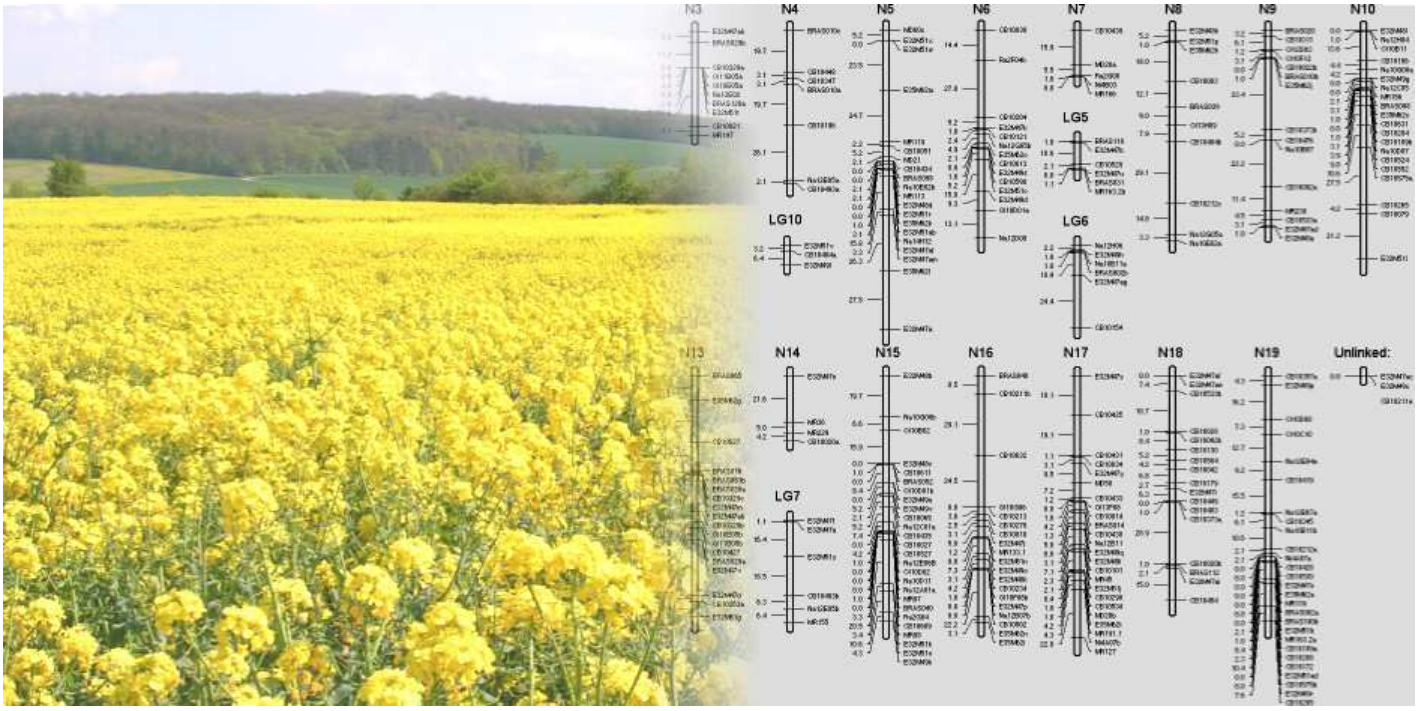

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Genetic Analysis of Heterosis in Rapeseed (*B. napus* L.) by QTL Mapping

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D7

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To my wife Dessy, for her patience

To my family for their moral support

To Milena for her valuable advices and for always being there in a difficult momen

Contents

1. Introduction

1.1 Current Status of Rapeseed Production

One of the most spectacular plant breeding achievements in the last 40 years has been the quality improvement of the former rapeseed cultivars. The modifications from high to zero erucic acid content of the oil and from high to low glucosinolate content of the meal have led to a status change in the crop: from low to high quality for both oil and meal. With the aim to emphasize this improvement the term 'canola' has been introduced with reference to zero erucic acid and low glucosinolate (double-low) cultivars. The successful development of the 'canola' quality opened new avenues on the food and feed markets worldwide and transformed the production of the five principal vegetable seed oils, soybean, cotton seed, groundnut, sunflower, and rapeseed. From the fifth place in terms of production in the 1980s rapeseed production climbed up to the second place nowadays following soybean. Growing area and total production have developed rapidly on a global level for the last two decades. A similar trend of development was observed in Europe and Germany as well (Fig. 1).

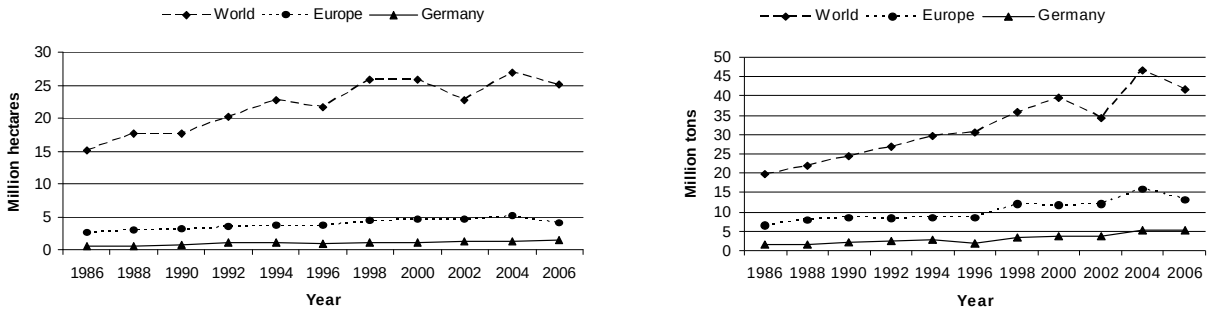


Fig. 1 Development of oilseed rape area (left) and production (right) in the world, Europe and Germany for the period 1986 – 2006 (data from FAO 2006)

The global rapeseed production in 2005 was 48.9 million tons of which 16.5 million (34%) were produced in Europe and 5.1 million (11%) in Germany (FAO 2006). Rapeseed comprises almost 75% of the oilseed production in the EU, followed by sunflower with 20%, soybean with 4% and cotton with 3%, (USDA FAS 2006). The vast majority of rapeseed is of higher-yielding, fall-planted cultivars, but there are still areas in the northeast (Poland, the Baltic states) that plant spring cultivars. Germany and France are currently the leading producers, together collecting 61% of the EU crop. USDA estimates are that Germany will produce 5.0 million tons in 2006/07, while France is estimated to produce 4.7 million tons. In 2005, the EU-25 has recorded its all-time highest rapeseed harvest, reaching 15.5 million tons

(USDA FAS 2006). The production increase was mainly due to a rise in the area sown but also attributed to the apparent trend in Europe towards higher yielding hybrid varieties. The hybrid breeding distinctly indicates that despite the predominantly self-fertilizing mating system and the relatively low out-crossing rate, heterosis can be put into use and plays an important role in rapeseed breeding in Germany. In 2006/2007 the use of hybrid varieties in Germany reached 65%, followed by Hungary 50%, Austria 40%, France 30%, UK 20%, and Poland 20%.

1.2 Hypotheses to Explain Heterosis

The heterosis phenomenon, on which hybrid breeding is based, is the superior performance of F_1 hybrids produced by a cross between genetically distant homozygous parents, to their midparent value or to the value of the better parent. While the practical application of heterosis in plant breeding is quite successful in many crops through the development of hybrid varieties, the basic understanding of the phenomenon is not very advanced. It is apparent that heterosis is related to heterozygosity, but it is still an open question how the heterozygosity results in heterosis. Three main hypotheses exist that explain the basis of heterosis: dominance, overdominance, and epistasis hypothesis (Crow 1999; Goodnight 1999). The dominance hypothesis supposes that deleterious recessive alleles at different loci of one of the parents are nullified by the dominant alleles at the same loci contributed by the other parent in the F_1 hybrid. The overdominance hypothesis states that the heterozygous combination of the alleles at a single locus is superior to either of the two possible homozygous combinations at that locus. Epistasis assumes that epistatic interactions between different loci are the reason for heterosis. Additional hypotheses look on heterosis from biochemical, molecular or physiological points of view (Stuber 1999).

Currently, results from quantitative genetic experiments favour the dominance hypothesis (Crow 1999). On other hand, theoretical considerations and some observations indicate that epistasis could play a significant role in the expression of heterosis (Goodnight 1999). Epistasis, in genetic terms, best explains the metabolic balance hypothesis trying to understand heterosis from physiological level. In addition, results of multimeric enzyme studies are apparent example of true overdominance (Stuber 1999).

1.3 Genetic Variation and Evolution of Rapeseed

Oilseed rape (*Brassica napus* L.; genome AACC, 2n=38) is a relatively young species that originates through spontaneous hybridization between turnip rape (*B. rapa* L., syn.

campestris; genome AA, 2n=20) and cabbage (*B. oleracea* L.; genome CC, 2n=18). Its genetic diversity is small due to two main reasons: (1) rapeseed is of recent origin and extensive rapeseed cultivation and breeding started not more than 60 years ago and (2) the species has a narrow genetic base (Becker et al. 1995). Most probably in the evolutionary formation of rapeseed the spontaneous hybridization between *B. rapa* and *B. oleracea* has occurred several times and the amphidiploid *B. napus* has polyphyletic origin (Song and Osborn 1992). Nevertheless, the present breeding material of oilseed rape is derived from very few interspecific hybrid plants that occurred spontaneously some centuries ago in a limited geographical region. The gene pool of elite rapeseed breeding material has been further eroded by an emphasis on specific quality traits derived from strongly restricted genetic material. On the other hand a large genetic diversity is observed within the *B. oleracea* and *B. rapa* group, where the diploid nature and the natural cross fertilization coincide with the most ancient cultivation history in genus Brassica allowing for wide ecological dispersal and variable crop characteristics (Becker et al. 1999). This large genetic variability could be employed in increasing the genetic diversity of rapeseed by its artificial resynthesis from the two parental species. The relatively high extent of intergenomic recombinations between A and C genome in the amphidiploid rapeseed (Lydiate et al. 1995) additionally contributes to the origin of novel genotypes after the resynthesis (Seyis et al. 2003).

Resynthesized rapeseed has been used for a broad spectrum of purposes for about 70 years (for review see Engqist and Becker 1994). In most cases the resynthesized rapeseed has low fertility, winter hardiness, and oil content, as well as undesirable seed quality with high erucic acid and glucosinolate content. Nevertheless it carries valuable genes for specific traits. In breeding programs the resynthesized lines are backcrossed at least twice with adapted material, and hence the overall genetic diversity of rapeseed is only slightly increased (Becker et al. 1995). Engqist and Becker 1994 suggested overcoming some of the inferior characteristics of the resynthesized rapeseed while preserving the large genetic variability by recurrent selection without any backcrossing to breeding material. In this way a novel gene pool could be established that may broaden the genetic base of rapeseed breeding and could help to develop lines with a large genetic distance from the present breeding material, which will be of great use for the rapeseed hybrid breeding.

1.4 Heterosis in Rapeseed

The extent of heterosis in rapeseed has been analysed in a number of studies with widely varying results depending on the materials and crosses used. In spring rapeseed hybrids an average high parent heterosis of 30% with a range of 20 to 50% was observed, while for winter rapeseed hybrids an average high parent heterosis of 50% was reported, ranging from 20 to 80% as reviewed by McVetty (1995). In a literature review Becker (1987) reported midparent heterosis values for yield in the range of 4 to 63%. In 10 experiments with winter rapeseed, a mean heterosis of 30% was observed, while in 8 experiments with spring rapeseed the respective value was 27%. It was observed that the amount of heterosis depends on the environmental conditions as well (Diepenbrock and Becker 1995). Often heterosis is higher under unfavourable conditions and stress environments, which probably contributes to the better yield stability of hybrids observed by Leon (1991).

In winter rapeseed Röbbelen (1985) reported significant heterosis for seed yield in a topcross of the variety 'Jet Neuf' with 19 winter rapeseed breeding lines, tested at 3 locations. The hybrids outperformed the high parent 'Jet Neuf' by an average of 13%. Knaak and Ecke (1995) analysed heterosis in 22 hybrids, derived from a factorial crossing scheme of 10 winter rapeseed cultivars and breeding lines. The midparent heterosis observed ranged from -3.9% to 27.4% with an average of 16.9%. In the same study highly significant correlations of 0.72 and 0.73 (P = 0.01) were observed between the genetic distance, assessed with RFLP markers, and the heterrosis for grain yield and plant height, respectively. Similar results were reported by Diers et al. (1996) but for spring rapeseed.

The correlation between genetic distance and heterosis was already apparent in a study by Lefort-Buson et al. (1987), who observed an average heterosis for seed yield of 40% in hybrids derived from crosses between European and Asian inbred lines but only 12% and 16% in hybrids from crosses within these groups, indicating that crosses between the major genepools of rapeseed, spring, winter and Asian rapeseed (Diers and Osborn 1994) may exhibit considerably higher levels of heterosis than crosses within the genepools. Often the high levels of midparent heterosis observed in crosses between European and Asian genotypes are strongly overestimated because of the poor ecological adaptation of the exotic material. The large differences in flowering time, maturity and winter hardiness impede the use of crosses between the European and Asian genepools in hybrid breeding therefore efforts are made for the development of alternative heterotic genepools.

An alternative genepool in a long term perspective could be represented by resynthesized rapeseed (Becker and Engqvist 1995). It shows a large phenotypic and phenologic variability,

including many types that are well adapted to European growing conditions. Becker et al. (1995) assessed the genetic diversity between 17 resynthesized lines and a collection of 24 spring and winter cultivars, using isozyme and RFLP markers. Some resynthesized genotypes clustered among the winter forms but the majority were quite distinct from the conventional spring and winter rapeseed breeding material, indicating that the large phenotypic diversity observed in resynthesized rapeseed is a consequence of an equally large genetic diversity. In a comprehensive study of heterosis in hybrids of crosses including a resynthesized parent, Girke (2002) tested 88 hybrids produced by a topcross of 44 resynthesized lines with two male sterile testers. The observed heterosis ranged from 1.4 to 55.5% indicating that heterosis in crosses with resynthesized rapeseed genotypes can reach levels similar to those observed by Lefort-Buson et al. (1987) in crosses between European and Asian inbred lines. Heterosis in a subgroup of five highly heterotic hybrids derived from resynthesized rapeseed lines well adapted to German growing conditions was lower, but with a range from 25.7% to 35.8% and an average of 28.9% still higher than the heterosis observed by Knaak and Ecke (1995) in hybrids of winter rapeseed varieties and breeding lines. Based on these results a cross including a resynthesized parent was chosen for the development of the plant material used in the current study.

1.5 Genetic Linkage Map Construction in *Brassica* Species

In the last two decades a number of genetic maps have been developed for the diploid and amphidiploid species of the U triangle (U 1935). Most of the mapping activities in *Brassica* were focused on *Brassica napus* since it is of the greatest commercial importance (Landry et al. 1991; Ferreira et al. 1994; Uzunova et al. 1995; Parkin et al. 1995; Foisset et al. 1996; Lombard and Delourme 2001; Piquemal et al. 2005). However, considerable efforts were made in the genetic map construction in *Brassica oleracea* (CC) (Slocum et al. 1990; Camargo and Osborn 1996; Kearsey et al. 1996; Kianian and Quiros 1992; Landry et al. 1992; Quiros et al. 1994; Ramsay et al. 1996) and *Brassica rapa* (AA) (Chyi et al. 1992; McGrath and Quiros 1991; Song et al. 1991; Teutonico and Osborn 1994), whose diploid genomes are combined in the amphidiploid *Brassica napus* (AACC). The mapping of *Brassica nigra* (BB) was not so intensive (Truco and Quiros 1994; Lagercrantz and Lydiate 1996; Lagercrantz 1998).

To a great extent the quality of the linkage maps depends on the marker systems applied. The first maps were RFLP based, followed by RAPD, AFLP and SSR maps. The genetic maps often consist of a combination of different markers, which allows better genome

coverage (Lombard and Delourme 2001; Uzunova et al. 1995; Foisset et al. 1996). F_2 and backcross mapping populations are widely applied but doubled haploid populations are typical for *Brassica napus*, as it responds very well to tissue culture regeneration.

The first linkage map of *Brassica napus* was developed in a F_2 segregating population from a cross between two closely related spring rapeseed varieties. A total of 120 loci, covering 1413 cM from the rapeseed genome were localised using RFLP probes from a seedling-specific cDNA library (Landry et al. 1991). The pioneering mapping of *B. napus* genome by Landry was followed by numerous studies on the topic. Ferreira et al. (1994) compared maps derived from a doubled haploid and a F_2 population. Uzunova et al. (1995) constructed a genetic map, using RFLP and RAPD markers and mapped the first loci responsible for the variation of glucosinolate content. Using a cross between a resynthesized *Brassica napus* and a "natural" oilseed rape Parkin et al. (1995) identified the 10 A genome and 9 C genome linkage groups of *B. napus* and demonstrated that the nuclear genomes of *B. napus*, *B. rapa*, and *B. oleracea* have remained essentially unaltered since the formation of the amphidiploid species, *B. napus*. The N-nomenclature for linkage groups in rapeseed, widely used nowadays, is based on this map. Foisset et al. (1996) developed a linkage map in a doubled haploid population using isozyme, RFLP and RAPD markers and discussed the origins of the observed non-Mendelian segregations. Cheung et al. (1997) compared genetic maps of *Brassica napus* and *Brassica oleracea*. Lombard and Delourme (2001) constructed a framework consensus map of rapeseed by the integration of maps of three DH mapping populations, using isozyme, RFLP, RAPD, and AFLP markers. Covering 2429 cM of the rapeseed genome they demonstrated that the consensus approach allowed the mapping of a larger number of markers, obtaining a near-complete coverage of the rapeseed genome, filling gaps, and consolidating the linkage groups of the individual maps. In a more recent study Piquemal et al. (2005) developed a consensus *B. napus* map using 305 SSR markers and integrating maps of 6 F_2 populations. In a comprehensive study, over 1000 genetically linked RFLP loci in *Brassica napus* were mapped to homologous positions in the *Arabidopsis* genome on the basis of sequence similarity (Parkin et al. 2005). The observed segmental structure of the Brassica genome strongly suggested that the extant *Brassica* diploid species evolved from a hexaploid ancestor (Lagercrantz 1998; Parkin et al 2005).

1.6 Methods for QTL Mapping

With the development of dense molecular marker maps a new era started in quantitative genetics. The main prerequisites included the discovery of abundant, tissue and environment independent DNA markers and the invention of biometrical procedures for detecting the loci responsible for quantitative trait variation, called quantitative trait loci or QTL. QTL mapping encompasses procedures for identifying and locating QTL and analyzing the magnitude of their main genetic effects and epistatic interactions as well as their environmental interactions. This bridges the gap between the continuous phenotypic variation and the mechanisms of inheritance by dissecting a continuously varying trait in individual loci (Phillips 1998).

QTL mapping approaches can be classified in two major groups: single-QTL models and multi-QTL models (Liu 1998). In case no QTL interactions are considered, then the model is referred to as a single-QTL model, while in case of interaction testing a multiple-QTL model is used. Depending on the number of markers applied in the model, QTL mapping methods are divided into single marker analyses, simple interval mapping and composite interval mapping. QTL mapping methods can be based on different analytical techniques including one-way ANOVA, simple t-test, simple linear regression, multiple linear regression, nonlinear regression, log-linear model, likelihood functions, mixed linear models and the Bayesian approach (Jansen 1992; Jansen 1993; Lander and Botstein 1989; Wang et al. 1999; Zeng 1994).

1.6.1 Simple Interval Mapping (SIM)

The interval mapping (Lander and Botstein 1989) takes the fullest advantage of the whole genome molecular linkage maps. Instead of analysing one marker at a time, as in single-marker analysis, intervals between adjacent markers along a chromosome are scanned and a likelihood profile of a QTL position at any particular point in each interval across the entire genome is determined. The likelihood profile includes an estimation of the LOD score, which is the log of the ratio between the likelihood to detect a QTL in a particular position versus the likelihood of no QTL localised at that position. An alternative approach to interval mapping was developed by Haley and Knott (1992). It is based on multiple regression and produces very similar results to those obtained by the application of the maximum likelihood method, regarding the accuracy and power of detection. The main advantages of the multiple regression based analyses are the increased speed and simplicity. Using interval mapping it is possible to distinguish between tight linkage to a QTL with small effect and loose linkage to a QTL with large effect. Still the simple interval mapping cannot solve some problems. A major

problem is the influence of closely linked QTL. Two closely linked QTL with effects in the same direction can lead to a detection of a "ghost QTL" in the middle between the two QTL, while the two real QTL remain undetected. No QTL will be detected if the two QTL have opposite effects. Another disadvantage of this QTL mapping approach is that it does not control the background genetic variation, which may bias the QTL effect estimation and decreases the power of detection.

1.6.2 Composite Interval Mapping (CIM)

A step forward in improving the power of detection and resolving problems such as two QTL linked in coupling or repulsion phase was achieved by modifying the simple interval mapping to composite interval mapping (Jansen 1993; Zeng 1994). In case of CIM the background genetic variation is controlled by including in the statistical model partial regression coefficients of markers (cofactors), which are situated in genomic regions having influence on the trait. Absorbing the variation due to these loci by the cofactors leads to a decrease in the confidence interval of the QTL position, increasing the resolution of mapping and the power of detection. An often applied cofactors selection method is based on stepwise regression with specific F-statistic thresholds (f-to-enter, f-to-drop). Different algorithms such as multiple linear regression (Jansen 1993) and maximum likelihood methods (Zeng 1994) have been applied to composite interval mapping. All of the listed methods can only detect single locus QTL and estimate the genetic effects in separate environments or need previously adjusted mean data estimated across different environments. Therefore digenic epistatic interactions between QTL and QTLxE interactions could not be simultaneously analysed. Mixed linear models have recently been introduced to composite interval mapping (Piepho 2000; Wang et al. 1999). Due to the flexibility of the mixed linear model approach, the genetic model can be extended to more complex genetic situations such as genotype-environment interaction and epistasis (Wang et al. 1999).

1.7 Analysis of Heterosis at a QTL Level

QTL mapping has been increasingly used in recent years for studying heterosis. In a pioneering study of an analysis of heterosis by QTL mapping in maize Stuber et al. (1992) identified QTL for 7 agronomic traits, including grain yield. The prevailing mode of action of the identified QTL was overdominance. Testing all possible pair-wise combinations of markers linked to the mapped QTL no epistasis was found. In a later study of the same material by Graham et al. (1997) the major QTL for grain yield could be partitioned by fine

mapping into two dominant QTL linked in repulsion phase, revealing the seemingly overdominant action of the originally mapped QTL as pseudo-overdominance. In an attempt to break up repulsion linkages that might lead to pseudo-overdominance Lu et al. (2003) studied heterosis in maize for grain yield, grain moisture, stalk lodging, and plant height in a population derived from a F_2 population by three generations of random mating. Despite using this population 24 of 28 QTL for grain yield showed overdominance. On the other hand the majority of QTL for the rest of the traits showed only partial dominance. In a recent study of heterosis in maize Frascaroli et al. (2007) identified QTL prevailingly in the additive - dominance range for traits with low heterosis and predominantly in the dominance-overdominance range for traits displaying high levels of heterosis like plant height, seedling weight, number of kernels per plant, and grain yield. Testing epistasis by the mixed linear model approach (Wang et al. 1999) only few QTL were involved in digenic epistatic interactions.

In an experiment with rice Xiao et al. (1995) identified 37 QTL for heterosis in 12 quantitative traits in a cross between two subspecies. The majority of the QTL in this study displayed a dominant gene action, no overdominance was observed. By testing epistasis with the marker pairs linked to the identified QTL, no significant interactions were found. These results were in discrepancy with another study on rice carried out by Yu et al. (1997). The authors found that most of the QTL for yield and some of the QTL for yield related traits showed overdominance. Furthermore testing all possible pair-wise marker combinations a considerable number of epistatic interactions was observed. These results have been confirmed by a series of studies of heterosis in rice (Li et al. 2001; Luo et al. 2001; Mei et al. 2003; Mei et al. 2005). In these experiments, a wide spectrum of agronomic traits including plant height, grain yield and yield components were analysed by QTL mapping in RIL-populations, testcross populations with an independent tester and backcross populations. In all studies most of the QTL contributing to heterosis (~ 90%) appeared to be overdominant and a large number of background loci were involved in epistatic interractions associated with heterosis.

All studies mentioned above were carried out in maize, which is an outcrossing crop or rice, which is self pollinated. The molecular basis of heterosis in rapeseed, an allopolyploid and a partially allogamous crop are not investigated so far.

The main objective of this study was a genetic analysis of heterosis in rapeseed at the QTL level, including:

1. Identification of the levels of heterosis for agronomic important traits
2. Identification, localization and determination of the effects of QTL for heterotic traits (grain yield and yield components, early plant biomass, plant height), phenological traits (beginning of flowering, end of flowering and duration of flowering) and quality traits (oil, protein, glucosinolate, erucic acid and sinapine content).
3. Assessment of the contributions of different genetic effects, e.g. dominance, overdominance and epistasis to the expression of heterosis in rapeseed
4. Study of the correlation between molecular marker heterozygosity and hybrid performance
5. Identification of “hot spots” for QTL involved in heterosis

2 Materials and Methods

2.1 Materials

2.1.1 Plant Materials

The plant material consisted of a population of 250 doubled haploid lines (DHL) produced from a cross between the winter cultivar 'Express' and the resynthesized line 'R53', and the 250 corresponding test cross hybrids between the doubled haploid lines and the male sterile tester 'MSL-Express'. The development of the doubled haploid population from F_1 plants of the cross 'Express'x'R53' was commissioned to a company, Saaten Union Resistenzlabor GmbH, Leopoldshöhe, Germany, specialized on androgenesis and tissue culture in rapeseed and other crop plants. The two parents 'Express' and 'R53,' their F1 hybrid and the commercial hybrid 'Elektra' were used as checks in the greenhouse and field experiments.

The female parent 'Express 617' is an inbred line of the winter line cultivar 'Express'. 'Express', which is of 'canola' quality was released in 1993 by NPZ-Lembke®, Germany, and is still considered as one of the best line varieties with stable yield, very high oil content and *Phoma* tolerance. The male parent 'R 53' is a resynthesized line developed from an interspecific cross between *B. oleracea convar. capitata var. sabelica* and *B. rapa ssp. pekinensis*. The resynthesized parent is well adapted to German growing conditions, meaning that the high levels of midparent heterosis (~30%) observed in the studies of Girke 2002 are not due to an ecological misadaptation of the resynthesized line. 'R53' is very distinct from the commercially used breeding materials but nevertheless has a relatively high performance, which makes the crosses with this genotype particularly suitable for heterosis studies.

The male sterile version of 'Express' (MSL 007) and 'Falcon' (MSL 004) were provided by NPZ-Lembke. A specific property of this male sterility is that most rapeseed genotypes, used as pollinators, restore the pollen fertility of the hybrids.
'Elektra' is a recently developed hybrid variety released on the market in 2002 by NPZ-Lembke. It has been the highest yielding hybrid in Germany in recent years.

2.1.2 Chemicals and Enzymes

DNA extraction was carried out with Nucleon®PhytoPure® extraction kit (Amersham Biosciences GmbH, Freiburg, Germany). For DNA quantification Bio-Rad Fluorescent DNA Quantification Kit (Bio-Rad Laboratories CA, USA) was used. The restriction enzymes *Eco*RI and *Mse*I were purchased from MBI Fermentas GmbH and New England Biolabs, respectively. Taq polymerase from the type FIREPol® together with PCR reaction buffer and

$MgCl_2$ were from the company Solis Biodyne, Tartu, Estonia. T4 DNA ligase was a product of Promega GmbH. ATP was purchased from Sigma-Aldrich Chemie GmbH, while dNTPs were from Qbiogene. The consumables for the ABI PRISM® 3100 Genetic Analyzer: Hi-Di™ Formamide, GeneScan™-500 ROX™ size standard and POP6 polymer were delivered from Applied Biosystems, Foster city, USA. The producers and suppliers of all generally used laboratory chemicals are listed in Appendix 1

2.1.3 Microsatellite Primer Pairs

A total number of 622 SSR prier pairs were available (524 from private and 98 from public sources). Public SSR primer pair sequences were obtained at: http://ukcrop.net/perl/ace/search/BrassicaDB.

Public SSR primer pair sequences were predominantly developed at IACR Long Ashton and John Innes Centre (Lowe et al. 2004). The code (Ra, Ol, Na, and Ni) used in the names of these primer pairs and the derived markers is determined by the source species: *B. rapa, B. oleracea, B. napus, and B. nigra*, respectively. The primer pairs designated 'BRAS', followed by three digits and 'CB' followed by five digits have been developed by Celera AgGen, sponsored by an international consortium of private breeding companies. The primer pairs coded with 'MR' and 'MD' have been developed by the Institute of Agronomy and Plant Breeding of the University of Göttingen.

2.1.4 Oligonucleotide Adapters for AFLP Analysis

The oligonucleotide adapters were synthesized by MWG-Biotech AG, Ebersberg, Germany, and were provided as single stranded DNA:

*Eco*RI Adapter 1 (EA1)	5' – CTC GTA GAC TGC GTA CC – 3'
*Eco*RI Adapter 2 (EA2)	5' – AAT TGG TAC GCA GTC – 3'
*Mse*I Adapter 1 (MA1)	5' – GAC GAT GAG TCC TGA G – 3'
MseI Adapter 2 (MA2)	5' – TAC TCA GGA CTC AT – 3'

The single strands were mixed together EA1 with EA2 and MA1 with MA2. The solutions were heated to 56°C and left to cool down slowly in order to produce double stranded adapters.

Adapter sequences:

*Eco*RI Adapter	*Mse*I Adapter
5′-CTCGTAGACTGCGTACC-3′	5′-GACGATGAGTCCTGAG-3′
::::::::::::	::::::::::::
3′-CTGACGCATGGTTAA-5′	3′-TACTCAGGACTCAT-5′

2.1.5 Primers for AFLP analysis

One selective nucleotide- and three selective nucleotide *Eco*RI and *Mse*I primers were synthesized by MWG-Biotech AG, Ebersberg, Germany. Primer sequences are available at Appendix 4. To enable analyses on a capillary automated sequencer *Eco*RI primers with 3 selective nucleotides carried a fluorescent dye label, which was either 6-carboxy-fluorescine (FAM) or NED™.

2.2 Methods

2.2.1 Total DNA Extraction

For a genetic map construction a high number of genotypes have to be simultaneously analysed, which necessitate a fast and technically easy method for total DNA extraction. Depending on the necessary DNA quantity two different approaches were used. The two parents and their F1 hybrid were used for primer pair screening, thus a CTAB method for DNA extraction modified by Rogers and Bendich (1988) was applied for DNA extraction, which provides a high amount of DNA. The extraction of the doubled haploid lines from the mapping population was performed with Nucleon®PhytoPure® extraction kits, which are fast, technically easy and provide good quality DNA.

2.2.1.1 CTAB-Total-DNA Preparation

Chemicals used:

2 x CTAB-Buffer	100 mM	Tris-HCl (pH 8.0)
	20 mM	EDTA
	1.4 M	NaCl
	1%	PVP 40000
	2%	CTAB
10% CTAB-Solution	10%	CTAB
	0.7 M	NaCl
CTAB-Precipitation Buffer	50 mM	Tris-HCl (pH=8.0)
	10 mM	EDTA
	1%	CTAB

High Salt TE-Buffer	10 mM	Tris-HCl (pH=8.0)
	1 mM	EDTA
	1 M	Na Cl
1 x TE-Buffer	10 mM	Tris-HCl (pH=8.0)
	1 mM	EDTA

- Grind to fine powder in liquid nitrogen 5-10 g fresh or deeply frozen leaf material
- Transfer the powder into 250 ml Erlenmeyer flask
- Add 10 ml warm (65°C) 2 x CTAB-buffer
- Add Proteinase K to a final concentration of 100 µg/ml
- Incubate at 65°C for 30 min in a water bath. Agitate by shaking gently.
- Transfer the solution to a 50 ml centrifuge tube, add 1 volume of Chloroform/Isoamylalkohol (24:1), and shake slowly
- Centrifuge at 12,000 rpm for 10 min at 4°C (Sigma centrifuge 4K 10, Rotor Nr. 12166)
- Transfer the aqueous upper phase in a new centrifuge tube
- Add 1/10 volume 10% CTAB-solution
- Extract once more with 1 volume Chloroform/Isoamylalkohol
- Transfer the aqueous upper phase to a new centrifuge tube
- Add 1 volume warm (65°C) CTAB-precipitation buffer
- Centrifuge at 12,000 rpm for 10 min at 20°C to pellet the precipitated CTAB-DNA complex
- Dissolve the DNA pellet in 2 ml high salt TE buffer at 65°C
- Precipitate the DNA with 2 volumes of cold 96 % Ethanol at -20°C overnight
- Centrifuge with 12,000 rpm for 15 min
- Wash the DNA pellet with cold 70% Ethanol to remove excess salts
- Centrifuge with 12,000 rpm for 5 min
- Dry the pellet in an exsiccator
- Dissolve the pellet in 1 ml TE-buffer

2.2.1.2 DNA Extraction with Nucleon®PhytoPure® Extraction Kit

The DNA extraction was carried out with midi-prep kits, starting with 1 g fresh or deeply frozen leaf material, following the manual provided with the kit.

2.2.2 DNA Concentration Measurement

The DNA concentration was measured with a Bio-Rad VersaFluor™ Fluorometer (Bio-Rad, CA, USA) according to the manufacturer's manual using Bio-Rad Fluorescent DNA Quantification Kit (Bio-Rad, CA, USA) The Fluorometer detects the fluorescence of the complexes built from the binding of DNA molecules and the fluorochrome bisbenzimide (Hoechst 33258). This dye is highly DNA specific and does not bind to RNA molecules, thus RNA residues do not effect the DNA quantification. Due to this advantage the fluorometer technique provides more precise measurements compared to spectrophotometric methods.

2.2.3 Simple Sequence Repeat (SSR) Analysis

SSR (Litt and Luty 1989) analyses were carried out following the M13-tailing PCR technique (Schuelke 2000). Instead of a fluorophore dye, each forward primer carries an eighteen nucleotide long tail with the following sequence:

5'-TTTCCCAGTCACGACGTT-3'

The tail is complementary to a 23 nucleotides long fluorescently labelled M13-universal primer:

5'-AGGGTTTTCCCAGTCACGACGTT-3'

The PCR reaction starts with the forward- and reverse-primer and the amplified products carry the tail. Such tail carrying sequences are templates for the M13-universal primer. The products, resulting from an amplification primed with the M13-universal primer and the reverse primer, are fluorescently labelled and can be detected due to the fluorescence after exiting the fluorophore tag with a light having specific wavelength.

The M13-universal primer was labelled with the fluorophores 6-carboxy-fluorescine (6FAM™), hexachloro-6-carboxy-fluorescine (HEX™) and NED™, which fluoresce in blue, green and yellow, respectively, after being excited by a laser beam. The absorption wavelength of 6FAM™, HEX™, and NED™ is 494 nm, 535 nm and 546 nm, respectively, while when the fluorophores return from the first excited state to the ground state they emit light with wavelength of 518 nm, 556 nm, and 575 nm, respectively. The emitted light is used for fragment detection.

PCR reaction mix:

1 x PCR buffer	10 x PCR buffer
2.5 mM	$MgCl_2$
0.2 mM	dNTPs
0.05 µM	M13-universal primer
0.05 µM	fPrimer (forward primer)
0.05 µM	rPrimer (reverse primer)
1 U	Taq-DNA polymerase
25 ng	Template DNA
Add H_2O to 20 µl	H_2O

A two step touchdown PCR program was used in a Biometrta T1 Thermocycler (Biometra GmbH, Göttingen, Germany):
95°C for 3 min
5 cycles 95°C for 45 sec; 68°C (-2°C/cycle) for 5 min; 72°C for 1 min
5 cycles 95°C for 45 sec; 58°C (-2°C/cycle) for 1 min; 72°C for 1 min
27 cycles 95°C for 45 sec; 47°C for 30 sec; 72°C for 1 min
72°C for 10 min
4°C ∞

The touch down technique was applied since it provides better conditions when a large number of primer pairs with similar but not identical annealing temperatures are used. The step of 72°C for 10 min. prevents the observation of unspecific one nucleotide differences between the amplified products, which are the result of desoxiadenosine addition by the Taq-polymerase at the end of the PCR products.

2.2.4 Amplified Fragment Length Polymorphisms (AFLP) Analysis

AFLP analyses were performed following the method of Vos et al. (1995), modified according to Kebede and Kopisch-Obuch (unpublished).

a) Restriction:

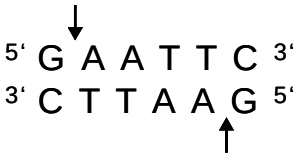
Total genomic DNA was digested with the enzymes *Eco*RI and *Mse*I by incubation at 37°C in a thermocycler for 90 minutes. The following reaction mix was applied:

250 ng	Genomic DNA
4 U	*Eco*RI

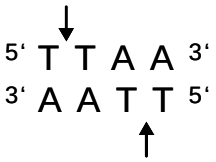
4 U	*Mse*I
1 x Restriction-ligation buffer	10 x Restriction-ligation buffer
Add H_2O to 30 µl	H_2O

The restriction-ligation buffer consists of 10 mM TrisAc, 10 mM MgAc, 50 mM KAc, DTT 5mM Dithiothreitol ($C_4H_{10}O_2S_2$) (DTT), The pH was adjusted to 7.5 with acetic acid.
*Eco*RI is a rare cutter, while *Mse*I cuts frequently. The recognition sites of the enzymes are:

*Eco*RI



*Mse*I



b) Ligation

The following reaction mix was used:

30 µl	Restriction product
5 pmol	*Eco*RI Adapter
50 pmol	*Mse*I Adapter
1 U	T4 DNA Ligase
0.25 mM	ATP
1 x Restriction-ligation buffer	10 x Restriction-ligation buffer
Add H_2O to 40 µl	H_2O

The ligation was carried out in thermocycler using the lollwing program:

1) 37°C for 3h 10min
2) 33.5°C for 3min
3) 30.0°C for 3min
4) 26.0°C for 4min
5) 22°C for 15min
6) 4°C ∞

The reaction was optimised to provide best conditions for the T4 DNA Ligase, avoiding the possible synthesis of ‘false’ AFLP fragments.

c) Preamplification

The preamplification was carried out with primers having only one selective nucleotide, which allows the amplification of a large number of fragments. The restriction-ligation product diluted 1:5 with HPLC grade H_2O was used as a template DNA for the reaction. The following reaction mix was applied:

8 µl	Diluted restriction-ligation product
10 pmol	*Eco*RI-Primer E01
8.7 pmol	*Mse*I Primer M02
0.3 mM	dNTPs
1.5 U	Taq-DNA-Polymerase
1 x	10 x PCR-buffer
4 mM	$MgCl_2$
Add H_2O to 20 µl	H_2O

10 x PCR buffer consisted of 800 mM Tris-HCl, 200 mM $(NH_4)_2SO_4$, 0.2% w/v Tween-20. The pH was adjusted to 9.4 – 9.5 with HCl.

The preamplification took place in 8 tubes strips of 0.2 ml (ThermoStrips^{TM}) on a Biometra T1 Thermocycler, following the program:

94°C for 30s

20 cycles 94°C for 30s; 56°C for 30s; 72°C for 1min

72°C for 5 min

4°C ∞

The preamplification product was diluted 1:10 and stored at 4°C

d) Selective amplification

The selective amplification was carried out using primers with 3 selective nucleotides. Such primers anneal approximately to only 1 of 64 fragments to which an unanchored primer would anneal. The probability a forward and a reverse primer to anneal to the same fragment is 1 of 4096, which drastically decreases the complexity of the banding pattern and instead of a smear a scorable banding pattern is observed along the lane on the gel.

The following reaction mix was used:

6 µl	Diluted preamplificatin product
2 pmol	EcoRI-Primer + 3
7 pmol	MseI-Primers + 3
0.24 mM	dNTPs
0.6 U	Taq-DNA-Polymerase
1 x	10 x PCR-Buffer
4 mM	$MgCl_2$
Add $H_2O$to 20 µl	H_2O

10 x PCR buffer consisted of 800 mM Tris-HCl, 200 mM $(NH_4)_2SO_4$, 0.2% w/v Tween-20. The pH was adjusted to 9.4 – 9.5 with HCl.
The amplification was carried out in a Biometrta T1 Thermocycler, following the profile:
94°C for 30sec
94°C for 30sec; 65°C for 30sec; 72°C for 2min
12 cycles 94°C for 30sec; 64.2°C for 30sec (-0.7°C/cycle); 72°C for 2min
25 cycles 94°C for 30sec; 56°C for 30 sec; 72°C for 2min (+ 1 sec/cycle)
72°C 5min
4 min ∞

Six primer combinations were screened by the two parents and their F1 hybrid.

E32M47	E32M49	E35M62
E32M48	E32M51	E32M61

2.2.5 Fragment Analyses on the ABI PRISM® 3100 Genetic Analyzer

ABI PRISM® 3100 Genetic Analyzer is a multi-colour fluorescence-based DNA analysis system with 16 capillaries operating in parallel. The detection system is based on excitement and fluorescent measurement of the fluorophores integrated to the amplified products during the PCR reaction.

2.2.5.1 Fragment Analyses of SSR Products

The use of three different colours 6FAM™, HEX™ and NED™ provided the possibility for loading a mixture of three differentially labelled PCR products simultaneously in each capillary (triple loading). Up to six fold multi-loading was performed by mixing the three possible colours with two products per colour. Two products of the same colour were mixed if the loci amplified by different primer pairs produced alleles easily distinguishable by size.

Two µl of the mixed PCR products diluted 1 to10 were added to a loading mix of 12 µl Hi-Di™ Formamide and 0.027 µl GeneScan™-500 ROX™ size standard (Fig 2). The mixture was denatured for 2 min at 95°C in a thermocycler. The electrophoresis was carried out on the sequencer at 60°C and 15 kV for 1h using a POP6 polymer, 36 cm capillary arrays and 22 sec injection time. GeneScan™-500 ROX™ size standard is designed for sizing DNA fragments in the 35-500 nucleotides range and provides 16 single stranded labelled fragments.

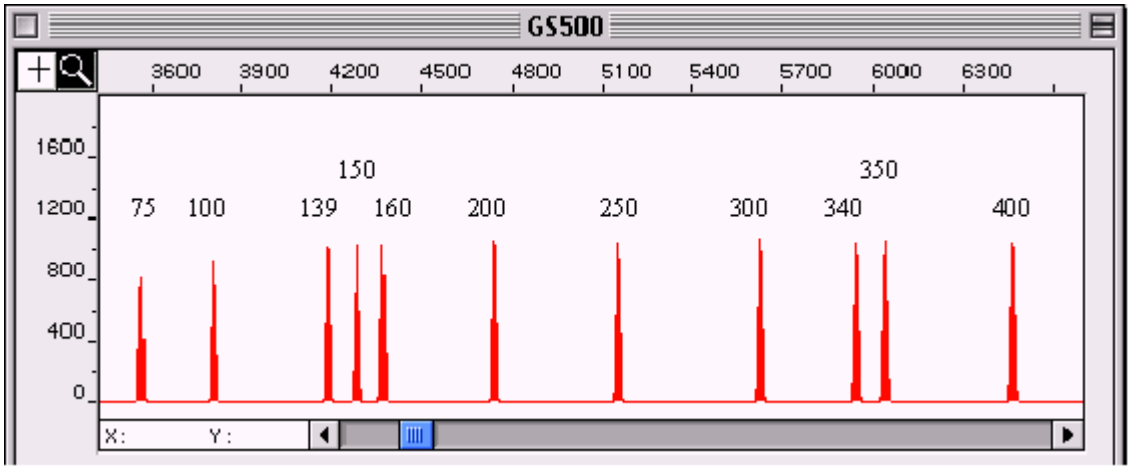


Fig. 2 GeneScan™-500 ROX™ size standard profile, including the 35 – 500 nucleotide range. The peaks of the fragments with sizes 35, 50, 450, 490 and 500 are outside the presented window.

2.2.5.2 Fragment analyses of AFLP Products

Fragment analyses of AFLP products were carried out without multi-loading. Only one colour was analysed at a time. The PCR product was diluted 1 to 5. The other conditions were the same as for the SSR analysis, as described in section 2.2.5.1.

2.2.6 Computer Analyses of the Raw Data Generated of the Fragment Analyses

GeneScan Software Version 3.7 (Applied Biosystems, Foster city, USA) was applied for the raw data analysis. The markers were scored using Genotyper Software Version 3.7 NT (Applied Biosystems, Foster city, USA). The same procedure was applied for SSR and AFLP analysis.

2.2 7 Bulked Segregant Analysis (BSA)

A bulked segregant analysis (Michelmore et al. 1991; Giovannoni et al. 1991) was used in order to saturate with markers a specific genomic region, which had not been covered with markers in the genetic mapping. Two bulks were formed, each consisting of 10 doubled haploid lines, which carried alleles from only one parent for both parts of the linkage group under study.

The following primer combinations were applied in the BSA:

E33M49	E41M50	E40M50
E33M59	E35M48	E38M50
E33M62	E35M50	E38M60
E40M62	E35M60	E41M47
E40M51	E33M48	E41M49
E38M62	E33M50	E35M47
E38M48	E33M61	E35M49
E41M48	E40M60	E35M59

2.2.8 DH-line Propagation and Testcross Development

The doubled haploid population was grown in 2004/2005 in isolation plots on the field at Reinshof breeding station for doubled haploid line propagation by selfing and for the development of test crosses by pollinating the male sterile testers ‘MSL-Falcon’ (004) and ‘MSL-Express’ (007). The isolation among the different genotypes was made with plots of *B. rapa*, which surrounded double rows of each doubled haploid line, flanked by double rows of the two testers. In this way the the pollinator of the male sterile testers could be only the doubled haploid line between them, which was selfed without bagging as no or few foreign pollen was able to cross the *B. rapa* isolation. A scheme of the isolation plots is presented in Fig. 3. The rows were 2.5 m long with 0.3 m between them. The distance between the plants within the row was 0.1 m. The width of the *B.rapa* isolation was 2.5 m at the upper and lower part of the plot and 1.8 m at both sides. The sowing was carried out by single seed drill in the period 23.08.2004 – 03.09.2004.

The agricultural procedures included:

1. Fertilizing
    - Nitrogen: 21.03.05 - 90 kg/ha N in form of KAS (lime, ammonium, saltpetre)
      11.04.05 - 80 kg/ha N in form of KAS (lime, ammonium, saltpetre)
    - Sulphur: 04.04.05 – 40 kg/ha S in form of Patentkali 225 kg/ha
2. Plant protection
    - Herbicides: 13.09.04 – 2 l/ha Butisan Top
    - Insecticide: 18.05.05 – 100 g/ha Karate Zeon
    - Fungicide: 20.04.05 – 0.7 l/ha Caramba

The soil type was of a very good quality including clay and loess (L2Lö), with "ackerwertzahl" of 84/82 according to the German soil quality rating ranging from 0 - very low to 100 – the best soil quality.

Manual harvest was performed from 13.07.05 to 20.07.05. After air drying for several days the harvested plants were threshed. The crosses with 'Falcon' (MSL 004) were not further used in the current study.

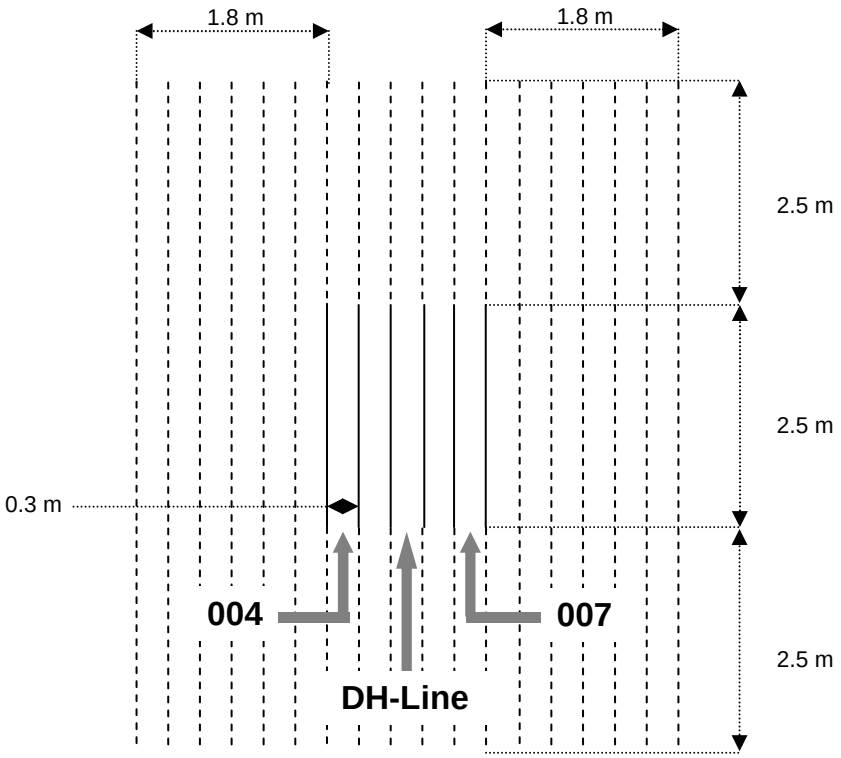


Fig. 3 Isolation plot for the production of test crosses. The DH-line is the pollinator of the male sterile testers 'MSL-Falcon' (004) and 'MSL-Express' (007). The dotted lines designate the isolation by rows of *B. rapa*

2.2.9 Greenhouse Experiment

For an assessment of early fresh shoot biomass heterosis a greenhouse experiment with four replications was carried out in the winter of 2005-2006. The plant materials used for the trial have been described in chapter 2.1.1. The sowing was done manually in square plots. Each plot consisted of 9 pots, 7x7 cm, filled with turf soil "T fein" type. The multi-array plots were organised on 12 tables. To adjust for light and temperature differences in the greenhouse an alpha lattice design 26-by-10 (Patterson and Williams 1976) was applied. Each of the checks, 'Express' and 'R53' for the doubled haploid lines, and F_1 ('Express' x 'R53') and 'Elektra' for the testcross hybrids, were replicated 5 times within the lattice. The doubled haploid lines and their corresponding hybrids followed the same alpha design but were grown on different tables in order to exclude competition between them. A single row of pots, sown with 'Express' or 'Elektra', was placed around the borders of each table with DH-lines or hybrids, respectively, to avoid border effects. To ensure that each plot contained the full number of plants at harvest, two seeds were sown in each pot. At first leaf stage one of the plants was removed, providing better growing conditions for the remaining plant. After the sowing and the plant emergence, the watering was done by spraying in order to moist the soil thoroughly. Subsequently, the watering was carried out by flooding to enable as even moisturising of the soil as possible. The flooding took place twice per week for 3 hours with 2 to 3 cm water depth. To ensure an adequate nutritive supply, NPK-Fertilizer "HaKaPhos blau" was added each time to the water in a concentration of 0.5 g/l. In addition to the day light each table was illuminated by two 400 watt SON-T-Agro sodium vapour lamps (Phillips, Netherlands) for 16 hours. The temperature was regulated according to the light and dark periods, 18 to 20°C and 15°C, respectively. Twenty nine days after sowing the shoot part of the plants was harvested and the fresh biomass was weighed as a bulk of each plot. The analysis was restricted only to the fresh biomass because of the very high correlation ($r \approx 0.95$) between the fresh and dry biomass observed by Abel (2006). The harvest was done in one day for all genotypes of a replication in order to prevent biomass differences due to different length of the growing period. The time span of the four replications is presented in Table 1.

Table 1 Greenhouse replications time table

Replication	Doubled haploid lines			Hybrids		
	Sowing	Harvesting	Growth duration[a]	Sowing	Harvesting	Growth duration
1	12.10.05	09.11.05	29	13.10.05	10.11.05	29
2	23.11.05	21.12.05	29	23.11.05	21.12.05	29
3	18.01.06	15.02.06	29	18.01.06	15.02.06	29
4	20.02.06	22.03.06	29	20.02.06	22.03.06	29

[a]The growth duration was estimated in days after sowing

2.2.10 Field Experiment

2.2.10.1 Experimental Design

The plant material used for the field trial has been described in chapter 2.1.1. It included the 250 doubled haploid lines and their corresponding test cross hybrids with the male sterile tester 'MSL-Express'. The parents 'Express' and 'R53' used as checks for the doubled haploid lines were replicated 5 times at each location (250 + 2 x 5 = 260). The F_1 hybrid (Ex x R53), and the commercial hybrid cultivar 'Elektra' were used as checks for the testcross population, also replicated 5 times at each location (250 + 2 x 5 = 260).

The experiment was carried out in the growing season 2005/2006, at 4 locations with different agroecological conditions (Reinshof, Deitersen, Rauischholzhausen and Grund-Schwalheim) and no replications per location. The lack of replications was due to limited field area at the breeding stations, as the experiment included 520 accessions grown in yield plots (about 10 m^2 each). The experimental design was a 26-by-10 alpha lattice (Patterson and Williams 1976). The properties of the plots at the different locations are described in Table 2. Specific for the design was that the doubled haploid lines and the hybrids were grown in parallel beds where each hybrid was placed at the same plot position in the second bed as the corresponding doubled haploid line in the first bed. Thus the two genotypes, which were compared in the subsequent analyses, were grown in as similar conditions as possible, excluding the competition between the lines and the more vigorous hybrids. A schematic representation is available in Appendix 2.

2.2.10.2 Location Properties

All locations were situated in Germany. Reinshof and Rauischholzhausen are the breeding stations of Georg-August-University of Göttingen and Justus-Liebig-University of Gießen, respectively. Deitersen, situated near Einbeck, belongs to the breeding stations of KWS, Einbeck, Germany and Grund-Schwalheim lying near Berstadt is a property of SW-Seeds, Sweden. Climatic, soil and plot properties of the locations are presented in Table 2.

Table 2 Properties of the locations of the field trial

Property	Reinshof	Deitersen	Rauischholz-hausen	Grund-Schwalheim
Altitude [m]	150	123	290	134
Long-term mean temperature [°C]	8.7	8.8	8.1	9.0
Long-term mean precipitation [mm]	645	644	603	–
Plot length [m]	7.5	10	8	6
Plot width [m]	1.50	1.75	1.50	1.80
Plot area [m^2]	11.25	17.50	12.00	10.80
Nr. of rows per plot	6	6	6	6
Between row distance [m]	0.25	0.21	0.25	0.30
Sowing density [seeds/m^2]	80	60	80	65
Soil Type	L 3 Al	L	L	L
Ackerwertzahl[a]	78/81	90	70	70
∅ Temperature [°C] 2005/2006	9.8	11.4	9.9	10.6
∅ Precipitation [mm] 2005/2006	629	445	499	615
Sowing date	24.08.05	31.08.05	30.08.05	05.09.05
Harvesting date	24-26.07.06	28-29.07.06	26-28.07.06	26-27.07.06

[a]German soil quality rating, ranging from 0 - very low to 100 - the best soil quality

2.2.10.3 Trait Evaluation

All agronomic traits evaluated are listed in Table 3. Near Infrared Reflectance Spectroscopy (NIRS) was carried out using the calibration equation `raps2001.eqa´ developed by Tillman (2005).

Table 3 Evaluated agronomic traits

Trait	Method of measurement	Units
Plant height (PH)	Measured at plot level from the soil to the up most plant part at end of flowering.	[cm]
Grain yield (GY)	Measured after combine harvesting as a bulk from the whole plot. Adjusted to 91% dry matter.	[dt/ha]
Thousand kernel weight (TKW)	Estimated as the average of 3 measurements of the weight of 100 seeds.	[g]
Seed/silique (S/Sil)	Estimated as a mean from 9 siliques. The first three siliques situated at the main raceme immediately above the first side branch were harvested from 3 randomly chosen plants per genotype and the seeds were counted.	none
Siliques/dm^2 (Sil/dm^2)	Estimated by the formula: Siliques/dm^2 = [GY per dm^2/(S/Sil*Single seed weight)]	none
Oil content (Oil)	Measured by near infrared reflectance spectroscopy (NIRS) as a percentage of 91% seed dry matter content.	%
Protein content (Pro)	Measured by near infrared reflectance spectroscopy (NIRS) as a percentage of 91% seed dry matter content.	%
Glucosinolate content (GLS)	Measured by near infrared reflectance spectroscopy (NIRS) in µmol/g seeds.	[µmol/g]
Erucic acid (C22:1)	Measured by near infrared reflectance spectroscopy (NIRS) in % of the total fatty acid content.	%
Sinapine (Sin)	Measured by near infrared reflectance spectroscopy (NIRS) in mg/g seeds	[mg/g]
Beginning of flowering (BF)	Measured as days from sowing to BF. BF is scored when 10% of the plants within the plot have at least one opened flower.	days
End of flowering (EF)	Measured as days from sowing to EF. EF is scored when at least 10 % of the plants within the plot have ceased to flower.	days
Duration of flowering (DF)	Measured as difference between EF and BF.	days

2.2.11 Data Analysis

2.2.11.1 Genotypic Analysis

2.2.11.1.1 Peak Patterns and Segregation Analysis

The raw output of the capillary electrophoresis was analysed by GeneScan software Version 3.7 (Applied Biosystems, Foster city, USA). Subsequently Genotyper software Version 3.7 NT (Applied Biosystems, Foster city, USA) was applied for marker scoring. In capillary electrophoresis the banding pattern typical for slab gels is replaced by a peak pattern. A presence of a band on the slab gel is equal to an observed peak in case of capillary electrophoresis. Based on the peak pattern in the segregating doubled haploid population it was possible to distinguish whether a particular SSR primer pair amplified one ore more loci and the allele sizes at the loci were determined. In case of a null allele in one parent a dominant marker was scored as presence or absence of the allele from the other parent. Uncertain peak patterns such as a very low peak or the detection of both parental alleles of a locus in a doubled haploid line were scored as missing data points. In case of stuttered peaks only the highest one was scored.

The scored data were organized in a file format compatible with MapMaker. Doubled haploid lines carrying 'Express' alleles were coded with 'E', while the rest of the lines were designated with 'R' for having 'R53' alleles. Missing data were represented with '-'.
The fit of marker segregation ratios to the 1:1 segregation ratio expected in a doubled haploid population was tested for each marker locus by a χ^2 test ($P = 0.05$).

2.2.11.1.2 Linkage Analyses and Map Construction

In the first step linkage analyses were carried out using all markers with a subset of 96 doubled haploid lines for the construction of a primary map. Subsequently the most evenly distributed markers were selected and extended to the rest of the lines of the doubled haploid population finishing with a total mapping population of 275 lines for the development of a framework map suitable for QTL mapping.

Linkage analyses were performed using MAPMAKER/EXP 3.0 (Lincoln et al. 1993). This version of the program allows analyses of F_2 and backcross populations. The segregation ratio in a DH-population is the same as in a backcross population, which allows the application of a backcross genetic model for linkage analyses to a doubled haploid population. The markers were grouped in linkage groups with a minimum LOD score of 4.0 and a maximum recombination frequency of 0.4. The LOD score is the logarithm to base 10 of the ratio of two

likelihoods, the likelihood of two markers being linked divided by the likelihood of the two markers segregating freely. A LOD score of 4.0 means that the linkage likelihood is 10^4 times higher than the free segregation likelihood.

Two point analyses, implemented in the 'group' command, were performed as the first mapping step. In this way the recombination values of all possible two marker combinations were estimated by the maximum likelihood method of Fisher and Balmakund (1928). Using these estimations markers were assigned to linkage groups according to the previously defined linkage thresholds (LOD $\geq$ 4.0 and r $\leq$ 0.4). To determine the correct marker order within the linkage groups exhaustive multi point analysis was performed by 'compare' and 'try' commands. Multipoint analysis takes into account the primary genotype data for all loci simultaneously, when determining map orders, marker distances, and map likelihoods. The recombination frequencies between the markers were transformed into map distances [cM] by the Kosambi mapping function (Kosambi 1944). The SSR markers showing the clearest banding patterns were used to construct the initial linkage groups, following the algorithm described above. All remaining SSR and AFLP markers were assigned stepwise to the initial maps by applying the 'try' command. Double crossover events were examined and the original scores rechecked for potential scoring errors. The order of the loci within the linkage groups was additionally verified by the 'ripple' command with a sliding window of 5 loci and a LOD score threshold of 2.0.

Based on shared markers the newly established map was aligned with four previously constructed maps (Lowe et al. 2004; Piquemal et al. 2005; Sharpe and Lydiate, unpublished data; Uzunova et al. 1995, extended version of the map), which allowed the linkage groups to be designated according to the 'N' nomenclature of Parkin et al. (1995).

2.2.11.2 Phenotypic Data Analyses

For statistical analysis of phenotypic data PLABSTAT Version 3A (Utz 2003) was used by the LATTICE procedure, which calculates adjusted mean values and provides a list of the incomplete block effects. The statistical model for a lattice design implemented in PLABSTAT Version 3A is:

$$Y_{ijk} = \mu + r_i + b_{ij} + g_k + e_{ijk,}$$

where Y_{ijk} is an observation of genotype k in block j of a replication i; μ is the general mean; r_i is the effect of replication i; b_{ij} is the effect of block j in replication i; g_k is the effect of genotype k; e_{ijk} is the error of observation Y_{ijk}.

The test for outliers implemented in PLABSTAT follows a modification of the method of Anscombe and Tukey (1963) that is based on the detection of extreme residuals. The list of detected outliers was examined and for the outliers with the highest standardized residual (Utz 2003) the data were checked for errors. In a second step, the analyses were repeated with the outliers considered as missing values. The analysis of variance (ANOVA) and adjusted means of this output were used in the subsequent analyses.

The broad sense heritability (h^2) was estimated as:

$$\hat{h}^2 = \frac{\hat{\sigma}_g^2}{(\hat{\sigma}_e^2 / r) + \hat{\sigma}_g^2}$$

Where $\hat{\sigma}_g^2$ designates the genotypic variance, $\hat{\sigma}_e^2$ shows the effective error variance and *r* is the number of replications. In the current study the environments were treated as replications and the error trerm includes genotype by environment interactions.

2.2.11.3 Datasets for QTL Mapping

The phenotypic data derived from the greenhouse and field experiments were organised in three different datasets, subsequently used separately for QTL mapping. The first dataset included the adjusted means of the doubled haploid lines, the second dataset consisted of the adjusted means of the test cross hybrids (DH-lines x 'MSL-Express') and the third set, referred to as midparent heterosis dataset (*MPH*-dataset), was estimated as the deviation (*Dev*) of the test cross hybrids (*TC*) from the midparent value (*MPV*) derived as the mean between the corresponding doubled haploid line (*DH*) and the tester 'MSL-Express' (*E*):

$$MPV = (DH + E)/2$$

$$Dev = TC - MPV$$

QTL, which contribute to heterosis are those detected with the *MPH*-dataset.

2.2.11.4 Estimation of Heterosis

The levels of midparent and high parent heterosis were estimated for the F_1 hybrid of the parents 'Express' and 'R53', referred to as F_1-heterosis, and for the testcross hybrids, referred to as average testcross heterosis. The latter was estimated as the mean of the heterosis values of all 250 test cross hybrids. The following equations were used for heterosis estimation:

1. MPV of 'Express' and 'R53':

$$MPV = (Ex + R53)/2$$

2. F_1 midparent heterosis:

$$MPH = F_1 - MPV$$

3. F_1 high parent heterosis:

$$HPH = F_1 - HP$$

4. $\overline{MPV}$ of DH-lines and 'MSL-Express':

$$\overline{MPV} = \frac{\sum_{i=1}^{250}\left[\left(DH_i + MSL - Ex\right)/2\right]}{250}$$

5. Average test cross midparent heterosis:

$$\overline{MPH} = \frac{\left(\sum_{i=1}^{250} TC_i\right)}{250} - \overline{MPV}$$

6. Average test cross high parent heterosis:

$$\overline{HPH} = \frac{\sum_{i=1}^{250}\left(TC_i - HP\right)}{250}$$

Where *MPV* is midparent value, *Ex* is 'Express', *MPH* is midparent heterosis, *HPH* is high parent heterosis, *HP* is high parent, *TC* is test cross hybrid, *DH* is doubled haploid line and *MSL-Ex* is 'MSL-Express'. $\overline{MPV}$, $\overline{MPH}$, and $\overline{HPH}$ are average midparent value, average midparent heterosis, and average high parent heterosis, respectively.

For testing the significance of heterosis values *t*-tests were applied.

2.2.11.5 QTL Mapping

The software QTLMAPPER version 1.0 (Wang et al. 1999) was used for QTL mapping. The program allows simultaneous interval mapping of both main effect and digenic epistatic QTL in RIL, DH or BC populations. It is based on a mixed linear model and performs composite interval mapping using cofactors. The model implemented in the program can be expressed as:

$$y_k = \mu + a_i x_{A_{ik}} + a_j x_{A_{jk}} + aa_{ij} x_{AA_{ijk}} + \sum_f u_{M_{fk}} e_{M_f} + \sum_l u_{MM_{lk}} e_{MM_l} + \varepsilon_k \ ,$$

where y_k is the phenotypic value of a quantitative trait measured on the *k*th individual (*k*= 1, 2 … n); μ is the population mean; a_i and a_j are the main effects (fixed) of the two putative QTL (Q_i and Q_j), respectively; aa_{ij} is the epistatic effect (fixed) between Q_i and Q_j;

$x_{A_{ik}}, x_{A_{jk}}$, and $x_{AA_{ijk}}$ are coefficients of QTL effects with a sign according to the observed genotypes of the markers (M_{i-}, M_{i+} and M_{j-}, M_{j+}) and values determined by the test positions ($r_{M_i-Q_i}$ and $r_{M_j-Q_j}$); $e_{M_f} \approx N(0, \sigma^2_M)$ is the random effect of marker f with indicator coefficient $u_{M_{fk}}$ (1 for M_fM_f and -1 for m_fm_f); $e_{MM_l} \approx N(0, \sigma^2_{MM})$ is the random effect of the lth marker interaction (between marker K_l and marker L_l) with indicator coefficient $u_{MM_{lk}}$ (1 for $M_KM_KM_LM_L$ or $m_Km_Km_Lm_L$ and -1 for $M_KM_Km_Lm_L$ or $m_Km_KM_LM_L$); $\varepsilon_k \approx N(0, \sigma^2_\varepsilon)$ is the random residual effect. The inclusion of e_{M_f} and e_{MM_l} is intended to absorb additive and epistatic effects of background QTL to control any bias in the estimation of QTL effects (Li et al. 2001; Wang et al. 1999).

The QTL mapping included four main steps performed with the software. First markers with significant influence on the trait (cofactors) were identified screening the whole genome by stepwise regression. The regression analyses were based on single marker genotypes for putative main effect QTL and on all possible pair wise marker pairs for epistatic QTL. The applied threshold was P = 0.005. In the second step composite interval mapping was performed in the genomic regions (covering two marker intervals) identified in the first step. Detected putative main effect and epistatic QTL were kept fixed in the model to control the background variation by the random effects of the cofactors. The applied threshold probability was P = 0.005 equivalent to a LOD-score of 1.71 (Wang et al. 1999). In the third step genetic parameters (effects and test statistics) were estimated for the putative main effect and epistatic QTL in the regions with a LOD score higher than the applied threshold. Finally the percentage of the explained phenotypic variation was calculated for each detected QTL.

The genetic expectations of the parameters estimated with the above model differ according to the type of the mapping population and the input data. The three datasets described in chapter 2.2.11.3 provide different genetic effect information. The doubled haploid lines provide an estimate for the additive effects ‘a’. Genetic effects detected with the MPH-dataset represent dominance effects (-d/2), while for the testcrosses the estimated effects are a combination of both dominance and additive effects - (a + d)/2 and (a – d)/2 if the donor or the recurrent parent carries a dominant increasing allele, respectively. An additional assumption is that the average of the test cross performance is higher than the MPV (positive heterosis); otherwise the estimated effects will have the opposite sign. The coding of the genotypes in the program also influences the sign of the effect. In this study the effects were estimated as a substitution of an allele from the resynthesized parent with an ‘Express’ allele. The dominance effects presented in chapter 3 were estimated from the output of the

program (-d/2) multiplied by -2, while the output of the QTL mapping with testcross hybrid data –(a+d)/2 or (a-d)/2 were multiplied by 2.

In case of epistasis the estimated effect in the doubled haploid population equals 4 times additive x additive genetic interaction (4aa). The effects in the other two datasets are complex mixtures of all possible epistatic interactions: additive x additive, additive x dominance, dominance x additive, and dominance x dominance interaction. If two loci *A* and *B* are considered, then the genetic effect in the testcross population represents $aa_{AB} + dd_{AB} - ad_{AB} - ad_{BA}$, while the effects estimated with MPH-data are $dd_{AB} - aa_{AB} - ad_{AB} - ad_{BA}$. Derivation of the metric effects is presented in Appendix 3.

2.2.11.6 Relationship between Genome Heterozygosity and Trait Expression

The relationship between genome heterozygosity and the expression of traits was tested by regressing testcross and midparent heterosis values on the genome heterozygosity assessed from the genome ratio of the 250 doubled haploid lines. The genome ratio is a percentage of the total genome of a doubled haploid line, which originated from a single parent. In the current study the genome heterozygosity in each testcross hybrid equals the percentage of 'R53' genome in the corresponding doubled haploid line. The calculation of the genome part contributed by each parent was performed according to the following rules: if two adjacent markers carried alleles from the same parent then the region between them was considered as coming from this parent. If an interval was formed by markers carrying alleles from different parents, then half of the interval was considered to be from one of the parent and the other half was considered as contributed by the other parent.

3. Results

3.1 Marker Screening

From 622 SSR primer pairs screened 447 (71.8%) gave clearly scorable banding patterns. The remaining primer pairs either failed to amplify products or the banding patterns could not be scored unambiguously. From the 447 successfully screened primer pairs 213 (47.7%) showed polymorphisms between 'Express' and 'R53', the parents of the mapping population, resulting in 270 putative markers. The screening of 5 AFLP primer combinations resulted in the detection of 119 putative markers. In most of the cases microsatellite primer pairs amplified only a single locus or a polymorphic locus accompanied by a monomorphic fragment but 39 out of 213 primer pairs (18.3%) amplified more than one locus. The majority of them, 35, amplified two polymorphic loci, 3 resulted in the detection of three loci and 1 amplified products from four different positions in the genome.

3.2 Construction of the Genetic Map

3.2.1 Primary Mapping

In a first step a primary map was constructed using all markers identified in the marker screening. They were mapped in a subset of the mapping populaion, which consisted of 96 doubled haploid lines. The size of the linkage groups, the marker number, the density of the markers on the linkage groups, and the alignment with the reference maps are summarised in Table 4. Two of the SSR primer pairs, which had shown polymorphisms in the parent screening failed to produce unambiguous banding patterns in the subsequent mapping in the doubled haploid population. Additionally, in the mapping four markers showed linkage to markers on two otherwise apparently separate linkage groups. These "cross-linkers" were excluded from the map. The final number of SSR markers on the primary map was 243, with 122 (50.2%) of them having been scored as dominant markers. In 49 of the dominant SSR markers the "null" allele was from 'Express', while in the other 73 markers no allele of the resynthesized parent was amplified.

In total the primary map included 243 SSR and 120 AFLP markers, organised in 24 linkage groups, covering 1916 cM of the rapeseed genome. Three markers remained unlinked and one of the linkage groups consisted only of two cosegregating AFLP markers. Of the mapped markers 114 (31.4%), exhibited a significant deviation ($P = 0.05$) from the expected 1:1 segregation ratio. An excess of 'Express' alleles was observed in 79 markers (69.3%).

'R53' alleles prevailed in the other 35 markers (30.7%). The markers with skewed segregation were not randomly distributed throughout the rapeseed genome. With an exception of a few markers on linkage groups N1, N5, N6, N11, N12, N13 and N18 the majority of the markers with disturbed segregation were clustered on linkage groups N2, N3, N4, N7, N8, N9, N10, N14, N15 and N19.

Table 4 Map alignment, linkage group size, number of markers, and marker density per linkage group of the primary map

Linkage Group[a]	**Size [cM]**	**Marker No.**	**Marker density**	**Map1**[b]	**Map2**	**Map3**	**Map4**	**Total**[c]
N1	112.9	22	5.13	10	6	0	1	13
N2a	51.9	7	7.41	3	2	0	0	3
N2b	50.5	4	12.63	0	1	0	0	1
N3	101.4	20	5.07	3	10	0	1	12
N4	73.9	7	10.56	2	3	0	0	4
N5	141.9	20	7.10	1	2	2	1	6
N6	101.0	14	7.21	4	1	1	0	6
N7	24.3	7	3.47	2	3	0	0	5
N8	68.8	8	8.60	0	2	0	0	2
N9	94.6	16	5.91	2	4	1	0	5
N10	111.4	20	5.57	7	6	0	0	10
N11	108.3	24	4.51	6	5	0	0	10
N12	43.1	22	1.96	2	3	0	1	5
N13	133.7	23	5.81	6	8	0	0	9
N14a	55.7	13	4.28	0	3	0	0	3
N14b	45.2	11	4.11	0	2	0	1	3
N15	117.8	27	4.36	3	7	0	0	9
N16	119.1	19	6.27	4	2	2	0	6
N17	127.5	24	5.31	3	7	0	0	8
N18	108.4	17	6.38	5	5	3	0	11
N19	100.3	27	3.71	1	9	1	0	11
LG5	14.8	6	2.47	0	0	0	0	0
LG10	9.6	3	3.20	0	0	0	0	0
LG11	0.0	2	0.00	0	0	0	0	0
Total	**1916.1**	**336**	**5.28**	**64**	**91**	**10**	**5**	**142**

[a]N2a, N2b, N14a and N14b are two unlinked parts of linkage groups N2 and N14, respectively
[b]Map1, Map2, Map3, and Map4 show the number of shared SSR markers used for alignment of the new map with previously established linkage maps, Piquemal et al. 2005, Sharpe and Lydiate, unpublished data, Lowe et al. 2004, and Uzunova et al. 1995, extended version of the map, respectively
[c]Shows the total number of SSR markers with which the new map was aligned to at least one of the four reference linkage maps

The linkage groups were named according to the N-nomenclature (Parkin et al. 1995) after an alignment of the newly constructed map with already established SSR linkage maps (Lowe et al. 2004; Piquemal et al. 2005; Sharpe and Lydiate, unpublished data, Uzunova et al. 1995, extended version of the map). Each linkage group was aligned with the reference maps based

on more than 3 shared SSR markers (Table 4).The nineteen major linkage groups, N1 to N19, which represent the 19 chromosomes of the rapeseed genome included 352 (97.0%) markers. Two additional small linkage groups LG5 with 6 markers and LG10 with 3 markers, together covering 24.4 cM, could not be aligned to the reference maps or linked to the rest of the linkage groups of the new map. The map alignment indicated that linkage groups N2 and N14 were not thoroughly covered with markers and remained split into two parts, N2a and N2b, N14a and N14b, with the applied linkage threshold (LOD = 4.0 and r = 0.4). Even with a reduced linkage threshold of LOD = 3.0 the separate parts of the linkage groups remained unlinked.

3.2.2 Framework Map

The most evenly distributed markers from the primary map were used for the development of a framework map, suitable for QTL mapping (Fig. 4). The framework map consisted of 181 markers, including 131 SSR and 50 AFLP markers mapped with 275 doubled haploid lines. The markers formed 21 linkage groups, which covered 1798.4 cM of the rapeseed genome. Table 5 provides information on the size, number of markers and density of the markers on the linkage groups of the framework map.

For the construction of the framework map 275 doubled haploid lines were used, which increased the precision of the mapping in comparison to the primary mapping with 96 doubled haplid lines. The improved mapping precision resulted in the merging of the two parts of linkage group N2, although the interval between the former terminal markers, BRAS083 and MR144b, was large, 32.1 cM.

The clustering of the markers with skewed segregation remained similar to that of the primary map with two exceptions on linkage groups N6 and N12, where new clusters of markers with disturbed segregation formed. Six markers on linkage group N6 were skewed towards ‘R53’ allele, while for 5 markers on linkage group N12 the ‘Express’ allele was the more frequent one.

Table 5 Linkage group size, number of markers, and marker density per linkage group of the framework map

Linkage Group	Size [cM]	Marker No.	Marker density
N1	100.5	9	11.17
N2	141.6	11	12.87
N3	98.7	8	12.34
N4	77.9	6	12.98
N5	138.7	11	12.61
N6	85.2	9	9.47
N7	27.9	4	6.98
N8	50.8	7	7.26
N9	92.8	10	9.28
N10	103.0	9	11.44
N11	64.1	10	6.41
N12	33.3	7	4.76
N13	121.7	11	11.06
N14	117.4	10	11.74
N15	114.3	12	9.53
N16	104.9	10	10.49
N17	111.5	11	10.14
N18	82.4	10	8.24
N19	98.5	12	8.21
LG5	24.7	2	12.35
LG10	8.5	2	4.25
Total	**1798.4**	**181**	**9.94**

3.2.3 Bulk Segregant Analysis (BSA)

In an attempt to fill the gap between the two parts of linkage group N14, 24 AFLP primer combinations were tested in a bulk segregant analysis. Two bulks were formed, each consisting of 10 doubled haploid lines, which carried alleles from only one parent for both parts of the linkage group N14. In total 363 loci polymorphic between the two parents were observed. Nine (37.5%) of the 24 tested primer combinations showed a total of 14 markers polymorphic between the bulks. Nine of these markers were mapped to the terminal regions of N14a. One was positioned within the upper part of the linkage group, while the remaining 8 markers were mapped beyond the lower terminal marker of N14a, extending the linkage group with 22.1 cM. Five markers were mapped to linkage group N14b. All of them were placed not at the terminal regions but within the linkage group. The mapping of the new markers with 96 doubled haploid lines did not connect the two parts of linkage group N14. The extension of the terminal markers to a total number of 275 lines increased the mapping precision and resulted in a linkage between N14a and N14b with an interval of 23 cM between the markers E38M48_112E and E32M47_116R. The final map including all mapped markers is presented in Fig 4.

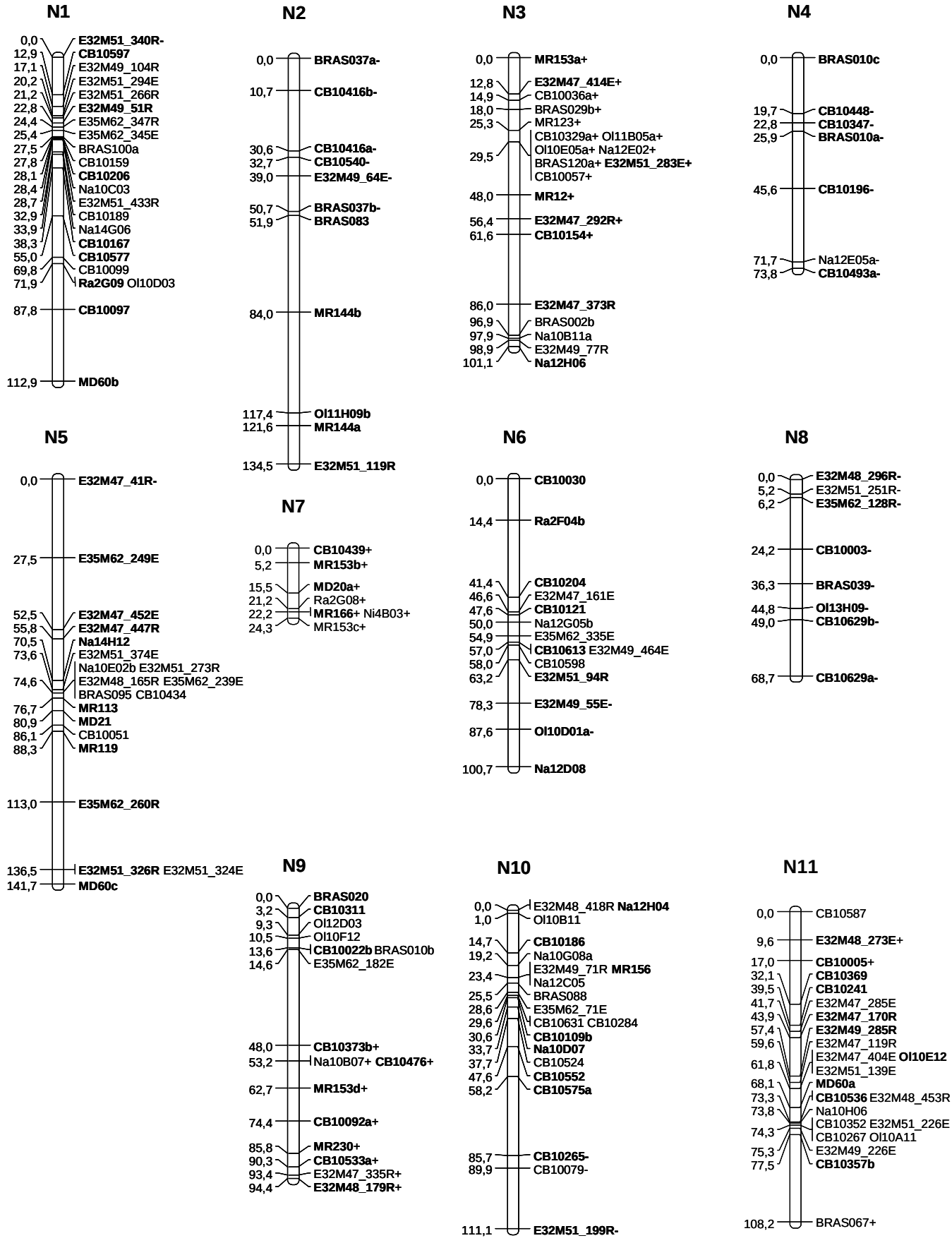
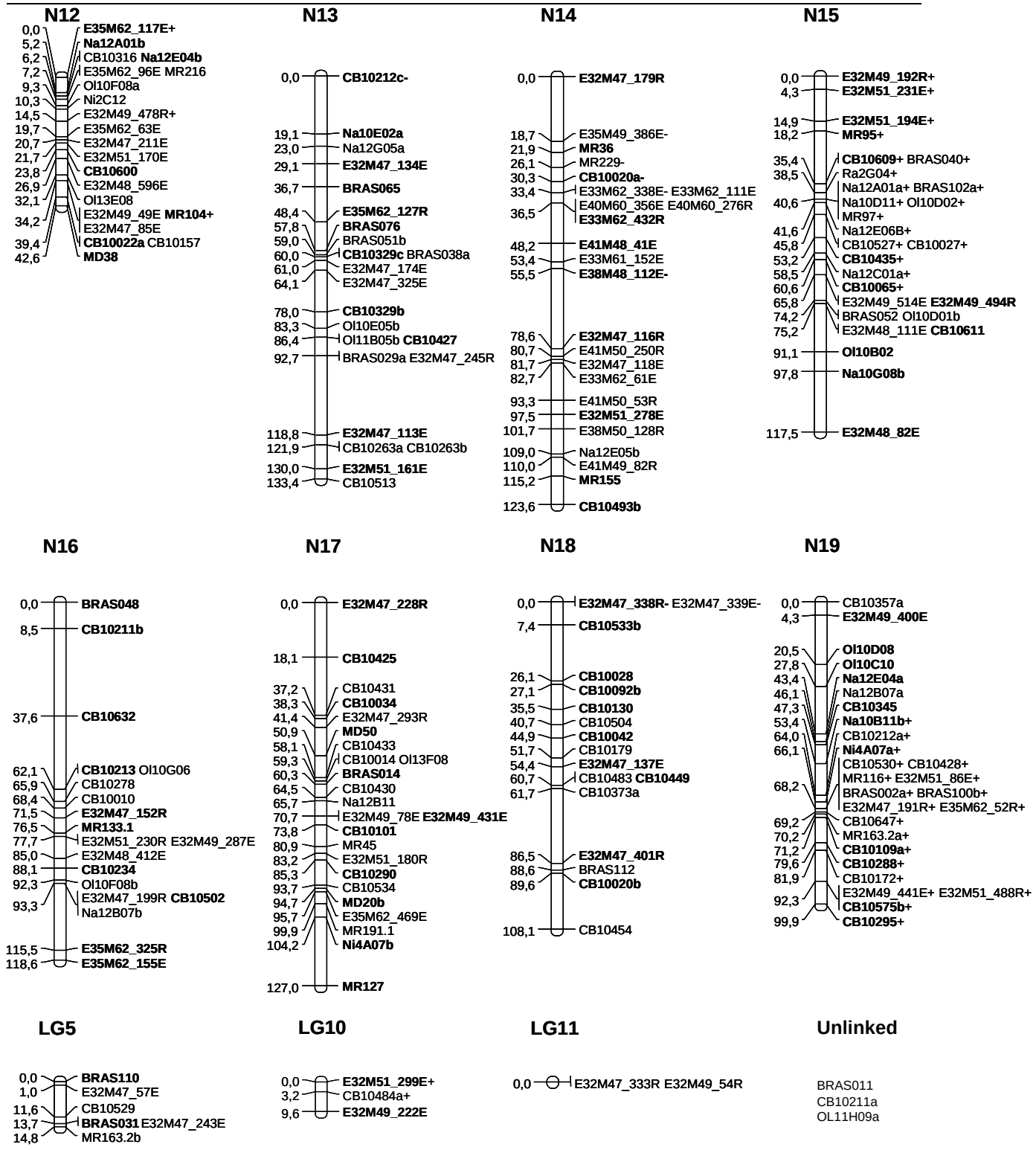


Fig. 4 Genetic linkage map of *B. napus* (cross 'Express'x'R53): Marker loci are presented in absolute positions from the beginning of the linkage groups in cM, estimated from the recombination frequencies assayed from 96 doubled haploid lines with the exception of the ❯



distances between the markers BRAS083 and MR144b on N2 and E38M48_112E and E32M47_116R on N14, which were estimated from the recombinations of 275 DH lines. The recombination frequencies were transformed in cM according to Kosambi mapping function. The markers in bold were chosen for the construction of the framework map. The markers deviating significantly from the expected 1:1 segregation ratio are designated with '+' if skewed towards 'Express' alleles, and with '-' if the 'R53' allele is the more frequent one.

3.3 Detection of Duplicated Regions in the Rapeseed Genome

Rapeseed is an amphidiploid species with a high level of similarities between the homoeologous A and C genomes, which explains the duplication of *B. napus* loci. Duplications occur also within the A and C genomes. RFLP markers proved to be very useful for studying the homoeology between different genomes in amphidiploids (Parkin et al. 1995; Sharpe et al. 1995). Duplications can be also studied using SSR primer pairs, which amplify more than one locus. The assessment of the level of duplications in the rapeseed genome was not a priority of the current study and the number of SSR primer pairs, which amplified more than one locus showing polymorphisms was not large enough for a comprehensive analysis, but the detected homoeologous regions between the A and C genome are an indication for the complexity of the rapeseed genome. A total of 39 primer pairs out of 213 (18.3%) amplified more than one polymorphic locus (see chapter 3.1). Based on them 42 duplicated regions were observed in the rapeseed genome. In 8 of the cases the duplications were on the same linkage group. This was most pronounced on linkage groups N2 and N13 where two internal duplications per linkage group were observed. Twenty two duplications were between homoeologous regions of the A and C genome. Four duplications were within the A- and 8 within the C genome. The complex relationships between chromosomes N3, N4, N9, N10, N12, N13, N14, N18 and N19 are presented in Fig. 5. In most of the cases the homoeologous regions were detected with not more than two markers (N3-N19; N10-N19; N4-N14b). More evident homoeology was observed between linkage groups N3-N13 and N9-N18. The homoeologous region between N3 and N13 included 4 markers covering 12 and 13 cM respectively. N9 and N18 shared 3 common markers which covered regions of 42 and 54 cM, respectively. It appeared that linkage group N19 consisted mostly of duplicated loci with an upper part corresponding to a region of N10, a middle region aligned to loci on N3 and N13, and a lower part corresponding to a region of N12.

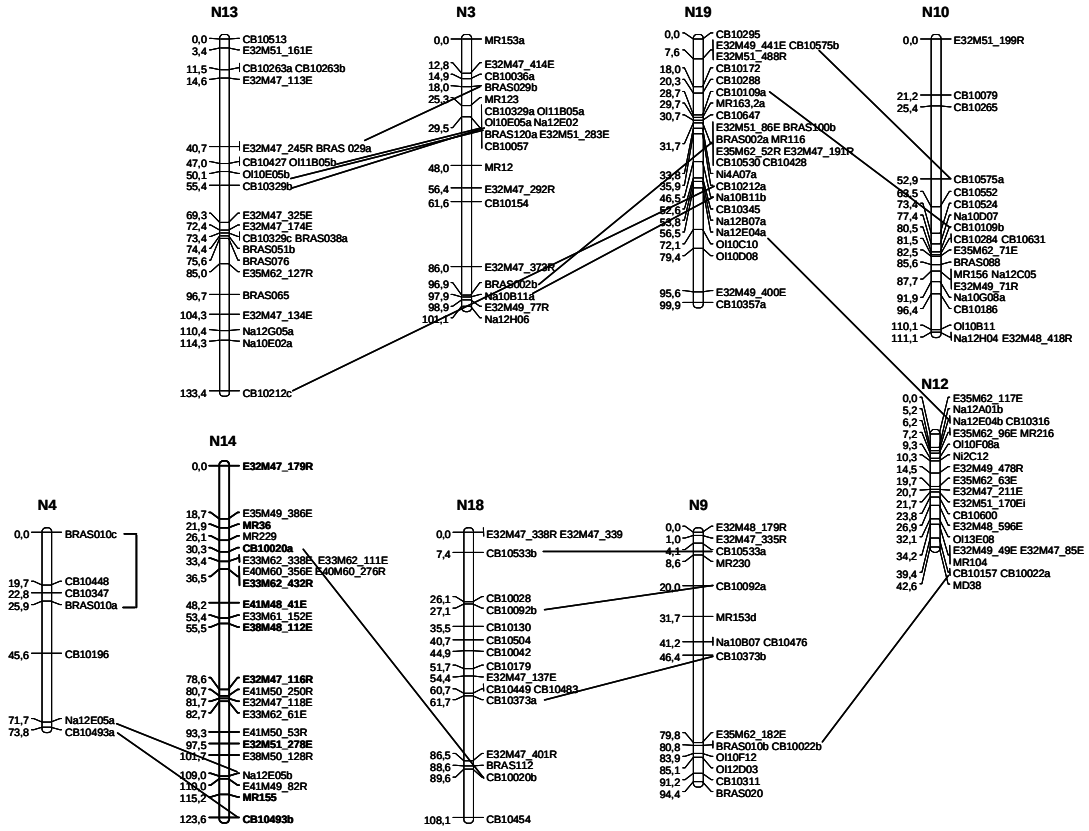


Fig. 5 Duplicated regions on linkage groups N3, N4, N9, N10, N12, N13, N14, N18, and N19. Lines connect corresponding duplicated loci

3.4 Analysis of Early Fresh Shoot Biomass

3.4.1 Heterosis for Early Fresh Biomass

Early fresh biomass of the doubled haploid and the testcross populations together with the parents 'Express' and 'R53', their F_1 hybrid, and the hybrid cultivar 'Elektra' used as checks, was measured in a greenhouse trial. Significant genetic variation was observed in the doubled haploid lines, the corresponding testcrosses and the midparent heterosis data. The heritabilities, genetic variances, and effective error means from the analysis of variance (ANOVA) of the alpha lattice are presented in Table 6.

For early plant biomass a highly significant midparent heterosis of 33% was observed in the F_1 hybrid and the high parent heterosis reached 15% (Table 7). The average testcross midparent heterosis was 12% (Table 8). This decrease in comparison with the F_1 heterosis was expected since the tester 'MSL-Express' is a male sterile version of the cultivar 'Express', used as a parent in the original cross for the development of the doubled haploid population. This leads to 50% less heterozygous loci in the test hybrids than in the parental F_1 hybrid, explaining the only half as large heterosis level. With only 5% the mean of the high parent heterosis of the testcrosses was of a lower magnitude but still statistically significant.

Table 6 Genetic variance, effective error mean and heritability of early fresh biomass in the doubled haploid lines, test cross hybrids, and midparent heterosis data

Poplation/Data set	$\hat{\sigma}_g^2$	$\hat{\sigma}_e^2$	$\hat{h}^2$
DH lines	6.01**	8.49	0.74
TC hybrids	0.99**	5.71	0.41
MPH	4.72**	13.59	0.58

$\hat{\sigma}_g^2$ genetic variance; $\hat{\sigma}_e^2$ effective error mean variance; $\hat{h}^2$ heritability

**Significant at $P = 0.01$

Table 7 F_1 and parental performance, midparent value and F_1 heterosis of early fresh biomass

				Heterosis (%)[a]	
Express ♀	R53 ♂	MPV	F_1	MPH	HPH
18.04	13.09	15.56	20.70	33.0**	15.0**

Early fresh biomass [g/plant];
[a]MPH and HPH: midparent heterosis and high parent heterosis. **Significant at $P = 0.01$

Table 8 Performance of 'Express', the doubled haploid population and the corresponding testcross hybrids as well as the average testcross midparent and high parent heterosis of early fresh biomass

	Mean of				
				Heterosis (%)[a]	
Express ♀	DH-Lines ♂	MPV	TC	MPH	HPH
18.04	17.79	17.91	20.03	12.0**	5.0**

Early fresh biomass [g/plant];
[a]MPH and HPH: midparent heterosis and high parent heterosis. **Significant at $P = 0.01$

3.4.2 Transgressive Segregation of Early Plant Biomass

A large number, 107 out of 250 (42.8%), doubled haploid lines showed higher biomass performance than the high parent. Forty six of them even outperformed the F_1 hybrid of the two parents. The transgressive segregation can be partially explained by the dispersal of alleles with positive and negative effects between the two parents. The distribution of early fresh biomass in the doubled haploid lines and the test cross hybrids are shown in Fig. 6.

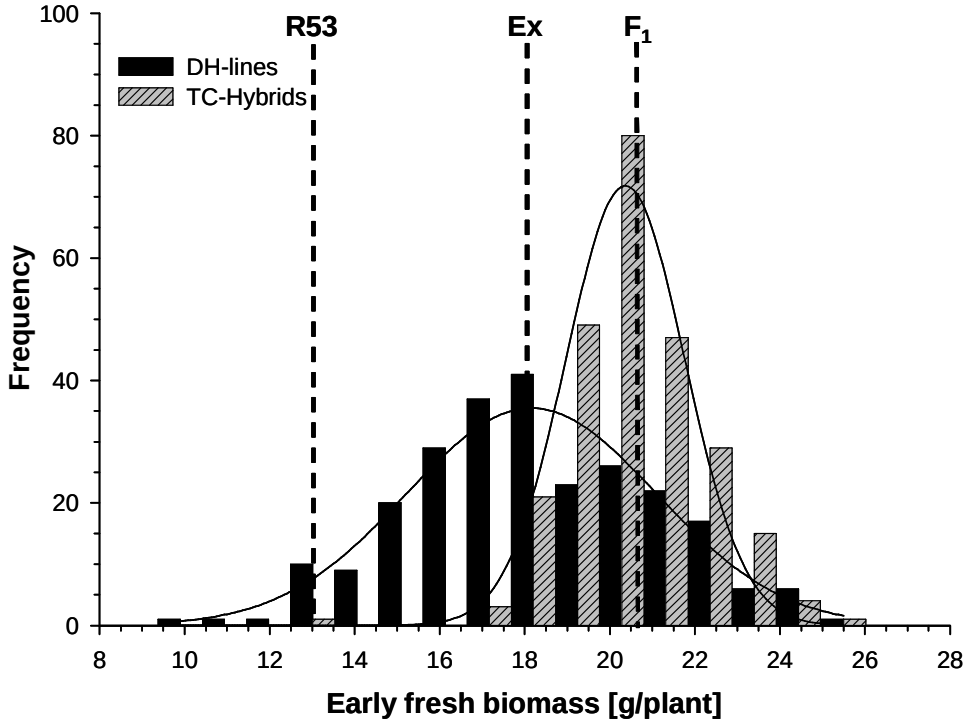


Fig. 6 Distribution of early fresh biomass in the doubled haploid lines and the testcross hybrids. 'Ex', 'R53' and 'F1' designate the mean values of 'Express', 'R53' and the F_1 hybrid

3.4.3 Relationship between Early Fresh Biomass and Genome Heterozygosity

The effect of genome heterozygosity on the midparent heterosis and on the performance for fresh biomass of the testcross hybrids was evaluated by regressing the midparent heterosis value and the trait value of each testcross hybrid on its percentage of genome heterozygosity. The Pearson correlation coefficient 'r', obtained from the regression analysis should reflect the importance of heterozygosity *per se* to the expression of a particular trait. The variation in early fresh biomass in the testcross hybrids showed no significant correlation to the heterozygosity level (r = 0.09). Midparent heterosis values were significantly correlated at $P = 0.01$ with the level of heterozygosity but only with a very low correlation coefficient, $r = 0.274$. The lack of correlation indicates that the overall genome heterozygosity alone had little effect on the trait expression.

3.4.4 Correlations between Line and Testcross Performance and Midparent Heterosis

The correlation analysis indicated that the variance in testcross performance was largely determined by the variation in heterosis instead of the variance in the performance of the corresponding doubled haploid lines (Table 9).

Table 9 Phenotypic correlation for fresh biomass between doubled haploid line values (DH), testcross hybridvalues (TC) and midparent heterosis values (MPH)

Coefficient[a]	Between DH & TC	Between MPH & TC	Between DH & MPH
r	0.265**	0.657**	-0.553**

[a]*r* – Pearson correlation coefficient, ** Significant at *P* = 0.01

With r = 0.657 the correlation between the testcross performance and the midparent heterosis was relatively high, while the correlation with the doubled haplod line performance at r = 0.265 was still significant but much lower. A moderate in magnitude, negative correlation was observed between the doubled haploid lines and the midparent heterosis.

3.4.5 QTL Mapping for Early Fresh Biomass

3.4.5.1 Analyses of Main Effect QTL

The results of the main effect QTL analyses of early fresh biomass using the three different datasets are summarised in Table 10. Four QTL significant at *P* = 0.005 were detected in the doubled haploid line population, which together explain 31.8 % of the phenotypic variance and 43% of the genotypic variance. In all cases the parent 'Express' contributed the increasing allele. A putative QTL significant at *P* = 0.05 was detected on linkage group N3, which coincided with a QTL with significant dominance effect. No significant QTL with additive effect was detected on linkage group N12, but the absolute value of the effect at this position was estimated from the other datasets (see chapter 2.2.11.5 and Appendix 3). The QTL mapping with the midparent heterosis values resulted in the localization of three QTL which explain a total of 14.8% of the phenotypic variance and 25.5% of the genotypic variance. In all three cases the dominance effects were positive, indicating that the allele increasing the trait was dominant. The dominance effect on linkage group N11 was estimated from the other datasets.

Table 10 QTL and their main effects detected in the doubled haploid line population (DH-Lines), the midparent heterosis values (MPH) and the testcross hybrid population (TC-Hybrids)

		DH-Lines				MPH				TC-Hybrids				
LG	**Interval**	**Pos[a] [cM]**	**LOD**	**Effect[b] [g/plant]**	**Vp[c] [%]**	**Pos[a] [cM]**	**LOD**	**Effect[b] [g/plant]**	**Vp[c] [%]**	**Pos[a] [cM]**	**LOD**	**Effect[b] [g/plant]**	**Vp[c] [%]**	**d/a[e]**
N3	E32M51_283E - MR12	47.5	3.1	0.35*	0.0	43.5	5.2	1.00***	8.2					2.9
N9	CB10476 - CB10373b									51.1	6.8	-0.76***	5.5	
N11	Ol10E12 - E32M49_285R	12.1	25.6	1.30***	21.1			(±0.49)		0.0	9.8	0.81***	6.3	0.4
N12	Na12E04b - Na12A01b			(±0.23)		34.2	4.0	0.77***	4.4	34.2	9.9	-0.54**	2.8	3.3
N13	E32M47_113E - E32M51_161E					141.4	4.5	0.54**	2.2					
N13	E32M47_134E - BRAS065	19.0	6.8	0.36**	1.6									
N13	CB10329b - CB10427	76.0	3.8	0.49***	3.0									
N19	CB10575b - CB10295	101.8	7.4	0.63***	6.1									

[a]Positions are measured from the beginning of the linkage group in cM
[b]* Significant at $P = 0.05$; ** Significant at $P = 0.005$; *** Significant at $P = 0.001$
The values in brackets are calculated from the effects at this locus detected with the other datasets
[c]Vp: Explained phenotypic variance [%]
[e]d/a: Dominance ratio

Three QTL were detected in the testcross population. They explain 14.6% of the phenotypic and 35.6% of the genotypic variance. Two of these QTL coincided with QTL detected with the other datasets but the QTL on linkage group N9 was detected only in the testcross population.

The degree of dominance was estimated under the assumption that coinciding QTL detected with different datasets represent one and the same QTL. Thus the QTL on N3 showed strong overdominance with a dominance ratio (d/a) of 2.9. Overdominance was observed also for the QTL on linkage group N12 with a dominance ratio 3.3. No QTL with significant additive effect was detected in the region on N13 where a QTL with dominance effect was located. The dominance effect in this region was 0.54 g/plant fresh biomass, evidently higher than the lowest additive effect – 0.36 g/plant, detected in the doubled haploid lines, which is an indication for overdominance in this region. The dominance ratio on linkage group N11 was 0.4 showing partial dominance.

3.4.5.2 Analyses of Epistatic Interactions

In the doubled haploid population a total of 23 loci involved in epistatic interactions in 12 digenic combinations were detected (Table 11). Two of these loci (on N11 and N13) showed significant additive effects. The epistatic interactions explained 34.1% of the phenotypic variance for early fresh biomass, which was slightly higher than the variance explained by the main effect QTL (31.8%). Epistatic effects were negative at 9 pairs of loci, indicating that recombination of the parental alleles increased early plant biomass, while at the other three pairs of loci parental allele combinations contributed positively to the trait.

In the QTL mapping with the testcross hybrids 10 digenic epistatic interactions were detected, which explained 38.1% of the phenotypic variation between the hybrids (Table 11). This was considerably higher than the 14.6% explained by the main effect QTL. Two of the 19 loci involved in epistatic interactions showed significant main effects as well.

Twenty loci involved in 11 pairwise epistatic combinations were detected with midparent heterosis data (Table 11). They explained 39.3% of the phenotypic variance. None of the loci involved in epistasis demonstrated significant main effects.

Table 11 Epistatic interactions for fresh biomass detected in the doubled haploid (DH) and the testcross hybrid (TC) populations and with the midparent heterosis values (MPH)

Set	LG	Pos[a]	LG	Pos	LOD	A_i[b]	A_j	AA_{ij}	$Vp_{(AAij)}$[c]
DH	N1	2.0	N11	12.1	25.6		1.30***	-0.33**	1.38
DH	N2	53.0	N12	23.0	4.2			-0.48***	2.95
DH	N3	47.5	N17	124.1	3.1			-0.39**	1.90
DH	N3	101.8	N14	102.1	3.3			-0.38**	1.80
DH	N4	19.1	N14	40.0	5.3			-0.52***	3.43
DH	N4	56.1	N7	28.2	4.8			-0.59***	4.27
DH	N4	72.1	N13	19.0	6.8		0.36**	-0.53***	3.48
DH	N5	78.3	N7	7.0	5.1			0.06***	4.49
DH	N6	0.0	N15	19.7	2.8			0.40**	1.97
DH	N10	29.6	N15	123.9	3.7			0.31**	1.22
DH	N11	43.2	LG10	8.0	5.9			-0.60***	4.46
DH	N18	9.2	N19	55.1	3.4			-0.47***	2.77
TC	N1	6.0	N14	12.0	3.6			0.39***	5.79
TC	N1	0.0	N18	56.9	3.4			-0.31***	3.73
TC	N3	59.3	N12	34.2	9.9		-0.27**	-0.27**	2.85
TC	N4	94.1	N8	44.4	5.5			0.33***	4.25
TC	N5	0.0	N11	0.0	9.8		0.41***	-0.32***	3.97
TC	N6	32.7	N19	72.9	3.5			0.32***	3.90
TC	N10	57.6	N17	47.9	3.1			-0.29***	3.11
TC	N13	6.0	N16	18.4	3.6			0.29**	3.24
TC	N13	21.0	N14	56.7	3.5			-0.29***	3.22
TC	N14	34.1	N15	111.9	3.8			-0.33***	4.04
MPH	N2	73.5	N14	2.0	2.3			0.29**	2.45
MPH	N4	2.0	N16	4.4	5.1			-0.35***	3.57
MPH	N4	24.9	N13	41.3	3.3			-0.33***	3.09
MPH	N4	60.1	N8	53.0	4.9			0.42***	5.22
MPH	N6	10.0	N11	69.6	3.2			0.36***	3.73
MPH	N6	10.6	N20	12.0	3.5			-0.37***	4.03

For abbreviations see page 47

Table 11/Continued from page 46

Set	LG	Pos[a]	LG	Pos	LOD	A_i[b]	A_j	AA_{ij}	$Vp_{(AAij)}$[c]
MPH	N6	59.4	N13	72.0	3.2			0.26**	1.98
MPH	N6	49.2	N18	63.4	3.1			-0.31***	2.89
MPH	N7	7.0	N9	107.4	3.2			-0.30**	2.71
MPH	N10	17.0	N11	2.0	4.3			-0.40***	4.71
MPH	N15	46.6	N16	55.9	5.0			0.41***	4.88

[a]Positions are measured from the beginning of the linkage group in cM
[b]A_i, A_j: main effects at loci i and j; AA_{ij}: epistatic interaction effect between loci i and j
* Significant at $P = 0.05$; ** Significant at $P = 0.005$; *** Significant at $P = 0.001$
[c]$Vp_{(AAij)}$: Explained phenotypic variation in [%] by the epistatic interactions

3.5 Analysis of Yield and Yield Components

3.5.1 Analysis of Variance and Heritability

The genetic variance and the heritability of grain yield (GY) and the yield components, thousand kernel weight (TKW), seeds per silique (S/Sil), and siliques per square decimetre (Sil/dm^2) are summarised in Table 12. Significant genetic variation was observed for all traits in the three different datasets. The heritability of grain yield and thousand kernel weight in the doubled haploid population was high, 0.83 and 0.91, respectively, while the heritability of seeds per silique and siliques per square decimetre was lower with 0.67 and 0.66, respectively. A trend to lower heritability in the testcross population was observed compared to the heritabilities in the doubled haploid population. This is understandable as the genetic variance of the testcross population was lower than that of the doubled haploid population because all testcross hybrids shared a common parent, while the environmental variance was of the same magnitude. The considerably high heritability of grain yield compared to other studies (Diepenbrock and Becker 1995) can be attributed to very high genetic variation observed in the doubled haploid population and the reliability of the experimental data, scored at four locations using yield plots organised in a specific design to control the environmental variance. The heritabilities calculated with the midparent heterosis values were similar to the heritabilities estimated in the doubled haploid population. A minor increase was observed for GY, while for TKW, S/Sil and Sil/dm^2 a slight decrease in MPH-data heritability was detected compared to the heritability in the doubled haploid population.

Table 12 Genetic variance, effective error mean and heritability of the doubled haploid lines, test cross hybrids and midparent heterosis data

Population/Dataset Trait[a]	$\hat{\sigma}_g^2$	$\hat{\sigma}_e^2$	$\hat{h}^2$
DH lines			
GY	30.14**	23.90	0.83
TKW	0.09**	0.03	0.91
S/Sil	8.20**	16.37	0.67
Sil/dm^2	40.10**	83.10	0.66
TC hybrids			
GY	4.44**	11.21	0.61
TKW	0.02**	0.02	0.72
S/Sil	1.83**	12.07	0.38
Sil/dm^2	7.17**	45.14	0.39
MPH data			
GY	33.37**	23.05	0.85
TKW	0.06**	0.05	0.80
S/Sil	5.83**	27.79	0.46
Sil/dm^2	41.01**	122.73	0.57

[a]GY, TKW, S/Sil, Sil/dm^2: Grain yield, thousand kernel weight, seeds per silique and siliques per dm^2, respectively; $\hat{\sigma}_g^2$ genetic variance; $\hat{\sigma}_e^2$ effective error mean variance; $\hat{h}^2$ heritability

**Significant at $P = 0.01$

3.5.2 Correlations between Grain Yield and Yield Components

Phenotypic correlations were estimated by the adjusted phenotypic means from four locations, making phenotypic correlations similar to genetic ones. The correlations between grain yield and yield components are presented in Table 13. The correlations estimated for the doubled haploid and testcross populations and the midparent heterosis data were different in magnitude but the sign of the correlations was the same. In all datasets the association between yield and seed weight was very low, indicating that variation in thousand kernel weight did not contribute strongly to variation in grain yield. Seeds per silique had a more evident but still low influence on yield. The number of siliques per square decimetre was the trait most closely correlated with yield, with $r = 0.611$ in the doubled haploid population. All correlation coefficients between yield components were negative, meeting the general opinion

that selection for one yield component only is not advisable since it would lead to a reduction in the other yield components.

Table 13 Correlations between yield and yield components estimated for the doubled haploid lines, the test cross hybrids and the midparent heterosis data

Dataset/Trait	GY	TKW	S/Sil
DH line population			
TKW	-0.136*		
S/Sil	0.263**	-0.480**	
Sil/dm^2	0.611**	-0.027	-0.466**
TC hybrid population			
TKW	-0.025		
S/Sil	0.121	-0.140*	
Sil/dm^2	0.371**	-0.074	-0.699**
MPH data			
TKW	-0.047		
S/Sil	0.116	-0.122	
Sil/dm^2	0.294**	-0.248**	-0.746**

*, **Significant at $P = 0.05$, $P = 0.01$, respectively. See Table 12 for abbreviations.

3.5.3 Analysis of Heterosis for Grain Yield and Yield Components

Table 14 and 15 present F_1 heterosis, the performance of the F_1 hybrid and its parents as well as their midparent value and the average testcross heterosis together with the means of the doubled haploid lines, their corresponding testcross hybrids and the mean midparent value of the doubled haploid lines with the tester 'MSL-Express'.

As expected, the most complex trait, grain yield, showed the highest level of heterosis – 30.0% F_1 heterosis and 13.0% average testcross heterosis. No significant high parent heterosis for yield was observed. The average testcross high parent heterosis was negative and statistically significant but with a very low magnitude. Thousand kernel weight did not show significant midparent F_1 heterosis, the average testcross midparent heterosis reached -1.2%, which was significant but very low, indicating that thousand kernel weight is unimportant for yield heterosis in the population under study. Seeds per silique exhibited positive midparent heterosis of 11.2% for the F_1 hybrid, in contrast to the expectations it reached 12.7% for the testcross hybrids. This was the only trait showing positive high parent heterosis but with low magnitude. The highest F_1 midparent heterosis of yield-determining traits was observed for

siliques per square decimetre – 19.0%, but the average testcross midparent heterosis was insignificant. Negative better parent heterosis was observed for F_1 and testcross hybrids but it was significant only in case of the latter. For the F_1 hybrid the yield heterosis was largely explained by the heterosis levels of seeds per silique and siliques per square decimetre, with the latter contributing stronger to heterosis. Seeds per silique was the only yield component contributing to the average testcross midparent heterosis.

Table 14 F_1 and parental performance, midparent value and F_1 heterosis

					Heterosis (%)[b]	
Trait[a]	Express ♀	R53 ♂	MPV	F_1	MPH	HPH
GY	47.61	23.53	35.57	46.19	30.0**	-3.0
TKW	4.44	4.21	4.32	4.29	-0.7^{ns}	-3.2**
S/Sil	25.41	23.97	24.69	27.46	11.2*	8.1^{ns}
Sil/dm^2	43.40	24.13	33.76	40.18	19.0**	-7.4^{ns}

[a]GY, TKW, S/Sil, Sil/dm^2: Grain yield [dt/ha], thousand kernel weight [g], seeds per silique and siliques per dm^2, respectively
[b]MPH and HPH: midparent heterosis and high parent heterosis. *, **Significant at $P = 0.05$, $P = 0.01$, respectively, ns – nonsignifivant

Table 15 Performance of 'Express', the doubled haploid population and the corresponding testcross hybrids as well as the average testcross midparent and high parent heterosis

		Mean of				
					Heterosis (%)[b]	
Trait[a]	Express ♀	DH-Lines ♂	MPV	TC	MPH	HPH
GY	47.61	32.16	39.88	45.04	13.0**	-5.0**
TKW	4.44	4.19	4.31	4.26	-1.2**	-5.0**
S/Sil	25.41	21.43	23.42	26.28	12.7**	2.6**
Sil/dm^2	43.40	37.57	40.48	40.98	1.8^{ns}	-7.9**

[a]GY, TKW, S/Sil, Sil/dm^2: Grain yield [dt/ha], thousand kernel weight [g], seeds per silique and siliques per dm^2, respectively
[b]MPH and HPH: midparent heterosis and high parent heterosis. *, **Significant at $P = 0.05$, $P = 0.01$, respectively, ns – nonsignifivant

3.5.4 Transgressive Segregation of Grain Yield and Yield Components

Figure 7 shows the distribution of phenotypic means for yield and yield components of the doubled haploid lines and their corresponding testcross hybrids. It is evident that the genetic variance in the testcross population was considerably lower, than in the doubled haploid population, which is attributable to the fact that test cross hybrids shared a common parent.

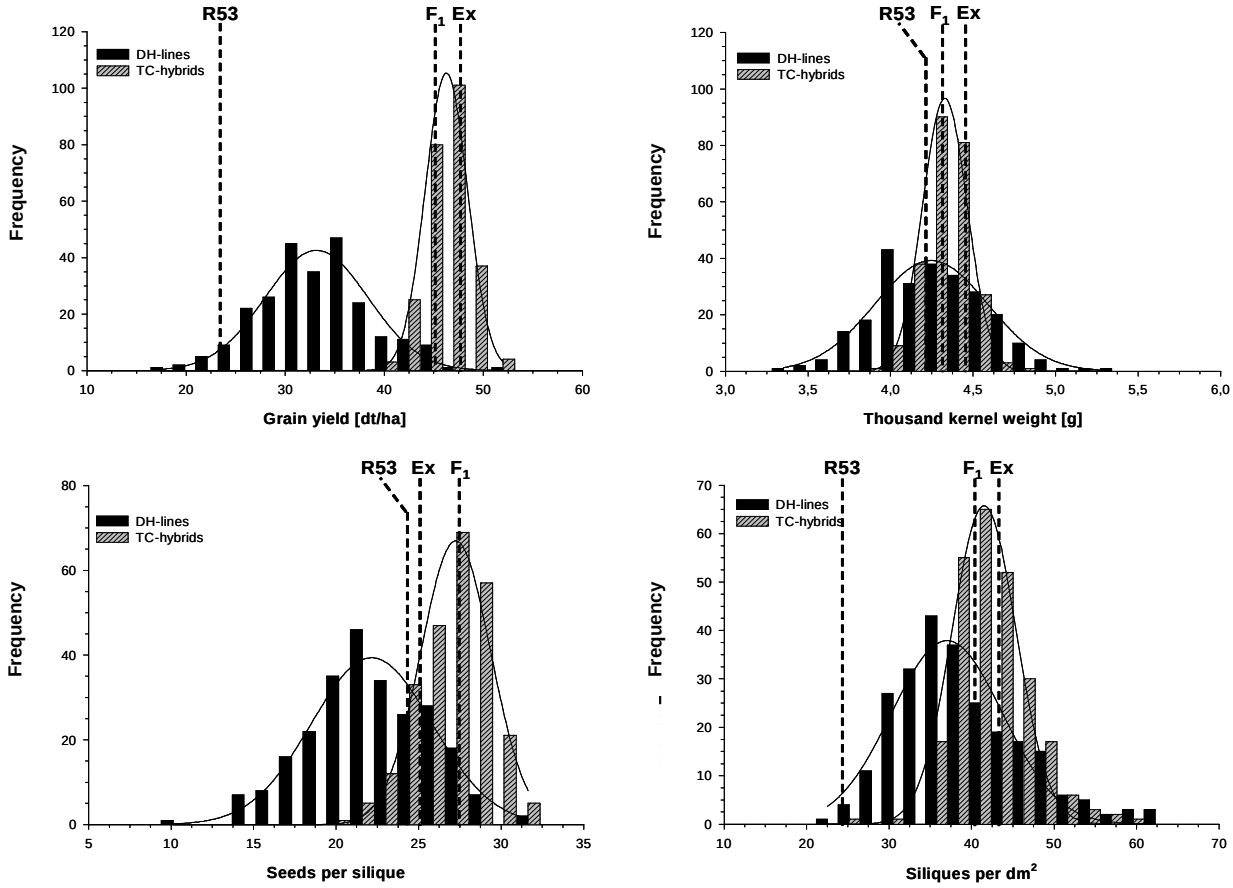


Fig. 7 Distribution of grain yield, thousand kernel weight, seeds per silique and siliques per square decimeter in the doubled haploid and testcross hybrid populations. 'Ex', 'R53' and 'F_1' designate the mean values of the parents 'Express', 'R53' and their F_1 hybrid.

For all traits evaluated, there were doubled haploid lines exceeding the phenotypic value of the high parent or the F_1 hybrid from which the doubled haploid population was developed. For grain yield, which showed the highest midparent heterosis, there still was one doubled haploid line with higher performance than the high parent and the F_1 hybrid. The fact that the doubled haploid line exceeded the phenotypic value of 'Express' was because of genetic reasons rather than environmental noise, as the field experiment was designed to control the environmental noise and experimental errors. The testcross hybrid distribution of thousand kernel weight and siliques per square decimetre overlapped completely with the doubled

haploid line distribution, which was expected as these traits showed no significant average testcross heterosis. Fifty seven (22.8%) doubled haploid lines were better than 'Express' for thousand kernel weight and for siliques per square decimetre 'Express' was outperformed by 54 lines (21.6%). In case of seeds per silique where significant F_1 and average testcross heterosis were observed, still 9 doubled haploid lines exceeded the phenotypic value of the F_1 hybrid and 36 (14.4%) were better than the parent 'Express'. As explained in chapter 3.4.2, the pronounced transgressive segregation is most probably a result of dispersal of positive and negative alleles between the parents as well as new formation of epistatic interactions.

3.5.5 Relationship of Grain Yield and Yield Components with genome heterozygosity

The effects of genome heterozygosity on midparent heterosis and on the testcross hybrid performance of yield and yield related traits are presented in Table 16. Except for the midparent heterosis of grain yield, no significant correlation was observed between the overall genome heterozygosity and testcross hybrid performance, indicating that overall genome heterozygosity alone had little effect on trait expression in the testcross hybrid population.

Table 16 Correlation coefficients (r) and coefficients of determinations (R^2) between genome heterozygosity and the trait value in the testcross hybrid population (TC-hybrids) and the midparent heterosis data (MPH)

Trait[a]	TC-hybrids		MPH	
	r	R^2	r	R^2
GY	-0.040ns	-0.002	0.287**	0.078
TKW	0.019ns	-0.004	0.045ns	-0.002
S/Sil	0.033ns	-0.003	0.044ns	-0.002
Sil/dm^2	-0.067ns	0.000	0.108ns	0.008

[a]GY, TKW, S/Sil, Sil/dm^2: Grain yield [dt/ha], thousand kernel weight [g], seeds per silique and siliques per dm^2, respectively
ns – nonsignificant, **Significant at $P = 0.01$

3.5.6. Correlations between Line and Testcross Performance and Midparent Heterosis Values of Grain Yield and Yield Components

The correlation analyses showed that the performance of individual testcross hybrids was determined by different factors for different traits under study. In case of seeds per silique and siliques per square decimetre the variation of the testcross hybrid performance was

determined predominantly by the variation of heterosis instead of the variation of the performance of their doubled haploid parents (Table 17). The correlation coefficients between midparent heterosis and testcross hybrid performance were 0.703 and 0.698 for seeds per silique and siliques per square decimetre, respectively, while the correlations for the same traits between doubled haploid lines and testcross hybrids was 0.243 and 0.202, statistically significant but low in magnitude. Opposite results were observed for thousand kernel weight. For this trait a higher correlation coefficient of 0.666 was detected for doubled haploid lines and test cross performance, while the correlation between the hybrids and midparent heterosis was lower with a correlation coefficient of only 0.238, which is consistent with the result that for thousand kernel weight average testcross heterosis was very low. The testcross performance for this trait was largely determined by the parental doubled haploid performance.

Table 17 Phenotypic correlation between doubled haploid line values (DH), testcross hybridvalues (TC) and midparent heterosis values (MPH)

Trait[a]	Coefficient[b]	Between DH & TC	Between MPH & TC	Between DH & MPH
GY	*r*	0.401**	0.354**	-0.715**
TKW	*r*	0.666**	0.238**	-0.566**
S/Sil	*r*	0.243**	0.703**	-0.519**
Sil/dm^2	*r*	0.202**	0.698**	-0.560**

[a]GY, TKW, S/Sil, Sil/dm^2: Grain yield [dt/ha], thousand kernel weight [g], seeds per silique and siliques per dm^2, respectively
[b]*r* – Pearson correlation coefficient, ** Significant at $P = 0.01$

For grain yield the correlation coefficients between doubled haploid lines and testcross hybrids and between midparent heterosis values and testcross hybrids were 0.401 and 0.354, respectively. Nearly equal correlation coefficients indicated that the variation of the doubled haploid line performance and the levels of heterosis of individual hybrids contributed to similar extent to the variation in the performance of testcross hybrids. The correlation between the doubled haploid lines and midparent heterosis data for all traits under study was negative and of moderate magnitude.

3.5.7 QTL mapping for Yield and Yield Components

3.5.7.1 Analyses of Main Effect QTL

The results of the main effect QTL analyses for yield and yield determining traits are summarised in Table 18 and Fig. 8.

Grain Yield: Six QTL with effects significant at $P = 0.005$ were detected in the doubled haploid population, which explained 32.7% of the phenotypic and 39.4% of the genotypic variation. Five QTL showed positive additive effects indicating that the parent 'Express' contributed the beneficial alleles, as expected since it is an elite variety. The effect of the QTL on linkage group N5 was negative, meaning that the allele of the resynthesized parent increased the yield. The QTL on linkage group N12 exhibited the highest additive effect and it alone explained 12% of the phenotypic variation.

Three QTL with effects significant at $P = 0.005$ and one additional putative QTL, significant at $P = 0.05$, were detected with the midparent heterosis data. Together they explained 18.1% of the phenotypic and 21.3% of the genotypic variation. Only one significant QTL was detected in the testcross population, which contributed 2.3% and 3.8% to the phenotypic and genotypic variance, respectively. A putative QTL with low effect, but congruent with the QTL detected at this locus in the other datasets was localised on linkage group N12.

The QTL detected simultaneously in the different datasets allowed an assessment of the degree of dominance of the QTL (Table 18). The QTL on linkage group N12 showing the highest additive effect, exhibited partial dominance with a dominance ratio (d/a) of 0.74. Partial dominance was also observed for the QTL on linkage group N19, while the QTL on N13 showed overdominance. The QTL on N6 was only detected in the midparent heterosis data, with a dominance effect of 1.07 dt/ha. This effect was higher than the lowest additive effect detected in the doubled haploid population, indicating overdominance for the QTL on N6. The QTL mapped on linkage group N3 with the testcross data most probably represents a sum of the additive and dominance effects, which were not large enough to be detected individually in the doubled haploid population or the midparent heterosis data.

Thousand kernel weight: Seven QTL with additive effects significant at $P = 0.005$ were mapped in the doubled haploid population, which explained 27.8% of the phenotypic and 30.5% of the genotypic variance. Three of these QTL on linkage groups N1, N7 and N12, respectively showed negative effects, indicating that the resynthesized parent contributed alleles for larger seeds. The remaining four QTL showed positive additive effects, meaning that the alleles for heavier seeds were from 'Express'. The dispersal of alleles with positive

and negative effect on thousand kernel weight between the two parents, observed on QTL level, explained the pronounced transgressive segregation detected on phenotypic level (see 3.5.4). Two putative QTL not significant at $P = 0.005$ but congruent with QTL showing significant dominance effects were mapped on linkage group N19. In addition the additive effect on linkage group N3 was estimated from the effects detected with the other datasets, as explained in Appendix 3 and chapter 2.2.11.5.

Three QTL with dominance effects were mapped with the midparent heterosis data. They explained 26.5% and 33.1% of the phenotypic and genotypic variance, respectively. The dominance effects of the QTL on linkage groups N1, N7 and N11 were calculated as described above. Two QTL showed positive dominance effects indicating that the allele increasing seed size was dominannt but the largest dominance effect on linkage group N19 was negative, partly explaining the small negative, average heterosis for thousand kernel weight in the testcross population (Table 15).

Four QTL detected with testcross hybrid data, explained 28.7% of the phenotypic and 39.9% of the genotypic variance. The two QTL on linkage groups N7 and N11 showed effects as large as the additive effects detected at these loci in the doubled haploid population, which is a hint for additivity. Most of the detected QTL for thousand kernel weight showed only additive effects, which is not surprising as the observed heterosis level for this trait was negative and very low (-1.2%). Nevertheless the 3 QTL with dominance effects mapped on linkage groups N3 and N19 showed overdominance, while the QTL on N1 exhibited partial dominance (Table 18).

Seeds per silique: Three QTL were mapped in the doubled haploid population, which explained 25.5% of the phenotypic and 38.1% of the genotypic variance. The two QTL on linkage groups N5 and N11 showed negative effects, meaning that the resynthesized parent contributed the increasing alleles. For the QTL on N9 the allele for more seeds per silique was inherited from ‘Express’. Only one QTL significant at $P = 0.005$ was detected with the midparent heterosis data, which explained 4.3% and 9.3% of the phenotypic and genotypic variance, respectively. An additional putative QTL significant at $P = 0.05$ was localised on linkage group N19 in congruency with a QTL with additive effect at this position. The dominance effect of the QTL on N5 was calculated as explained in Appendix 3 and chapter 2.2.11.5.

The QTL mapping in the testcross population resulted in the detection of two QTL on linkage groups N5 and N17, which explained 18.3% of the phenotypic and 48.2 of the genotypic variation. The QTL effect on N5 was of similar magnitude as the effect detected at

this locus in the doubled haploid population, indicating additivity. The calculated dominance effect at this position was close to zero (Table 18). The QTL on linkage group N17 represents the sum of the additive and dominance effects at this locus, which individually were below the power of detection in this mapping experiment. The dominance effects of the QTL mapped with the midparent heterosis data were lower than the additive effects at these positions, which indicated partial dominance with dominance ratios of 0.7 and 0.6 for the QTL on linkage groups N11 and N19, respectively.

Siliques per square decimetre: Seven QTL were detected in the doubled haploid population, which explained 32.7% of the phenotypic and 49.5% of the genotypic variance. In all cases except the QTL on linkage group N19 the additive effect was positive, meaning that the parent 'Express' contributed the increasing alleles. No QTL with dominance effects were identified, which was congruent with the insignificant levels of heterosis observed for this trait (Table 17). Only one QTL was detected in the testcross population, explaining 6.3% and 16.2% of the phenotypic and genotypic variance, respectively. No QTL with additive effect was detected at this position.

Table 18 QTL and their main effects detected in the doubled haploid line population (DH-Lines), the midparent heterosis values (MPH) and the testcross hybrid population (TC-Hybrids)

			DH-Lines				MPH				TC-Hybrids				
Trait[a]	**LG**	**Interval**	**Pos**[b]	**LOD**	**Effect**[c]	**Vp**[d]	**Pos**	**LOD**	**Effect**	**Vp**	**Pos**	**LOD**	**Effect**	**Vp**	**d/a**[e]
GY	N3	E32M47_414E - E32M51_283E									15.0	4.0	0.62**	2.3	
GY	N5	E32M47_41R - E35M62_249E	103.4	6.1	-1.08***	4.1									
GY	N6	Ra2F04b - CB10030					94.8	4.8	1.07***	3.2					
GY	N7	CB10439 - MR153b	4.0	5.0	1.39***	6.8									
GY	N12	Na12E04b - Na12A01b	34.2	13.5	1.85***	12.0	31.6	13.9	1.36***	5.3	34.2	2.0	0.50*	0.0	0.8
GY	N13	CB10427 - E32M47_113E	87.5	5.8	1.09***	4.2	78.0	13.9	1.83***	9.6					1.7
GY	N17	MR127 - Ni4A07b	16.0	2.6	0.78**	2.1									
GY	N19	E32M49_400E - CB10357a	0.0	5.8	0.99***	3.5	0.0	4.9	0.56*	0.0					0.6
TKW	N1	CB10597 - E32M51_340R	104.2	6.5	-0.10***	6.4			(±0.05)		114.2	6.3	-0.05***	2.7	0.5
TKW	N3	MR12 - E32M47_292R			(±0.03)		59.3	4.0	0.05***	3.9	66.5	6.9	-0.08***	8.2	1.7
TKW	N5	MR119 - MD21	68.6	4.5	0.06***	2.2									
TKW	N7	MR153b - MD20a	17.0	9.4	-0.10***	6.8			(0.00)		20.2	8.7	-0.10***	10.7	
TKW	N9	CB10022b - CB10311	98.6	4.3	0.05**	1.5									
TKW	N11	CB10536 - CB10357b	0.0	5.7	0.07***	3.3			(±0.01)		2.0	7.1	0.08***	7.1	
TKW	N12	CB10600 - E35M62_117E	27.0	8.5	-0.08***	3.8									
TKW	N16	MR133.1 - CB10234	87.3	6.0	0.08***	3.8									
TKW	N19	Ni4A07a - CB10109a	74.9	5.7	-0.04*	0.0	70.9	7.0	-0.10***	16.2					2.5
TKW	N19	CB10288 - CB10575b	101.0	4.3	0.03	0.0	101.1	2.4	0.06**	6.4					2.0

For abbreviations see page 58

Table 18/Continued from page 57

Trait[a]	LG	Interval	DH-Lines				MPH				TC-Hybrids				d/a[e]
			Pos[b]	LOD	Effect[c]	Vp[d]	Pos	LOD	Effect	Vp	Pos	LOD	Effect	Vp	
S/Sil	N5	MD21 - MR113	70.1	13.9	-1.38***	8.8			(±0.20)		63.2	7.9	-1.58***	12.5	0.1
S/Sil	N11	CB10536 - CB10357b	4.0	15.3	-1.59***	11.7	0.0	4.2	-1.06***	4.3					0.7
S/Sil	N17	E32M49_413E - BRAS014									57.7	3.8	1.08***	5.8	
S/Sil	N19	CB10345 - Na10B11b	56.5	7.1	1.05***	5.0	52.0	4.0	0.66*	0.0					0.6
Sil/dm^2	N3	E32M47_414E - E32M51_283E	27.0	4.1	1.84***	4.5									
Sil/dm^2	N3	MR12 - E32M47_292R	65.3	2.5	1.23**	2.0									
Sil/dm^2	N5	E32M51_326R - E35M62_260R	42.2	6.6	1.50***	2.9									
Sil/dm^2	N7	CB10439 - MR153b									0.0	5.0	2.22***	6.3	
Sil/dm^2	N11	MD60a - Ol10E12	9.5	6.5	1.66***	3.6									
Sil/dm^2	N12	Na12E04b - Na12A01b	34.2	15.0	3.28***	14.1									
Sil/dm^2	N16	CB10211b - CB10632	20.4	6.6	1.44***	2.7									
Sil/dm^2	N19	CB10345 - Na10B11b	58.5	4.2	-1.50**	2.9									

[a]GY, TKW, S/Sil, Sil/dm^2: Grain yield [dt/ha], thousand kernel weight [g], seeds per silique and siliques per dm^2, respectively
[b]Positions are measured from the beginning of the linkage group in cM
[c]* Significant at $P = 0.05$; ** Significant at $P = 0.005$; *** Significant at $P = 0.001$;
The values in brackets are calculated from the effects at this locus detected with the other datasets
[d]Vp: Explained phenotypic variance [%]
[e]d/a: Dominance ratio

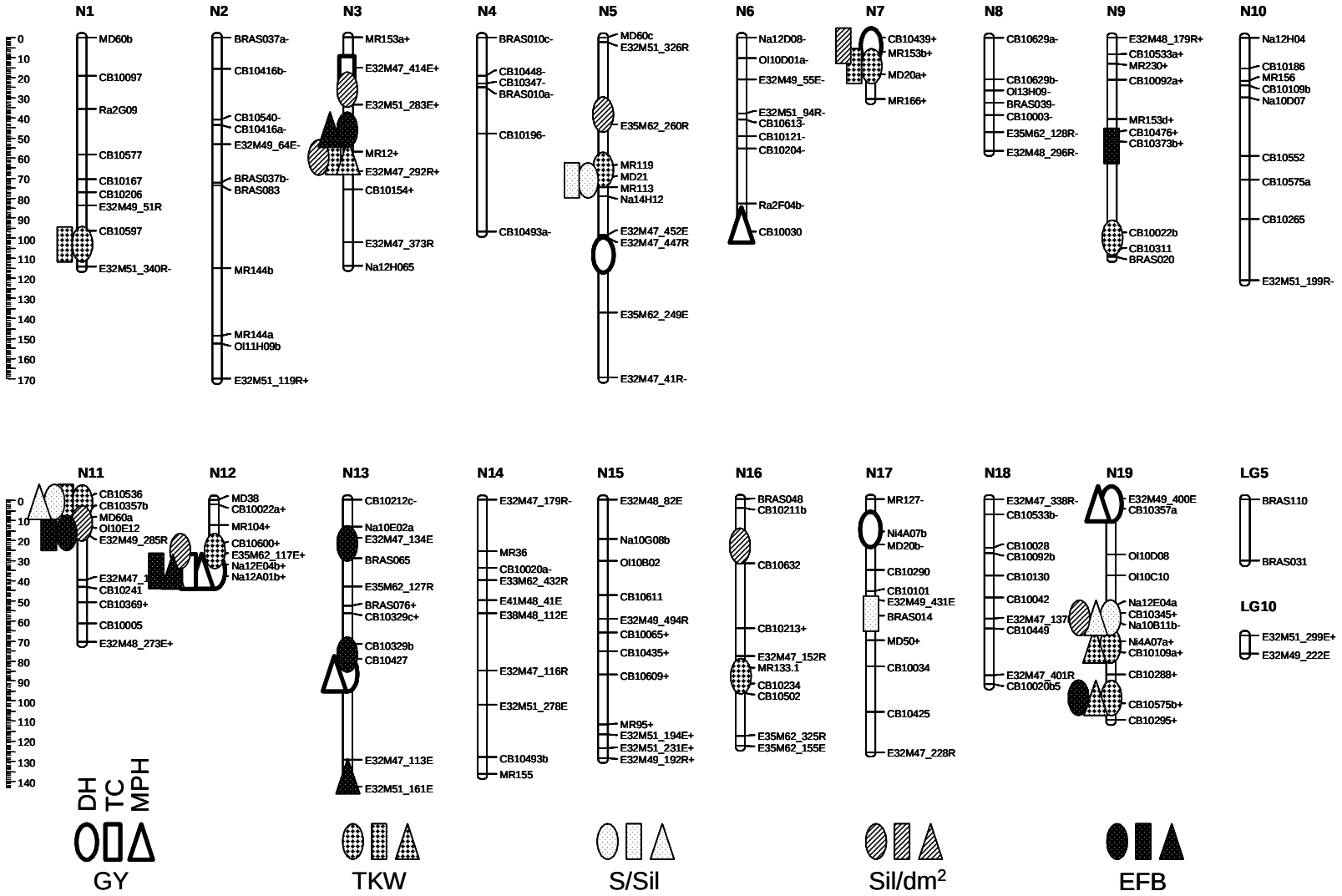


Fig. 8 Framework map of *B. napus* with QTL for grain yield (GY), thousand kernel weight (TKW), seeds per silique (S/Sil), siliques per square decimetre, and early fresh biomass (EFB). DH, TC and MPH are QTL detected in the doubled haploid population, the testcross population, and the midparent heterosis data, respectively. Marker positions are calculated from recombination frequencies according to Haldanes mapping function. For '+' and '-' signs at the end of the marker names see Fig 4.

3.5.7.2 Analyses of Epistatic Interactions

The results of the QTL analyses for epistasis are summarised in Table 19.

Grain yield: Six loci involved in 3 digenic interactions significant at $P = 0.005$ were detected in the doubled haploid population (Table 19). The epistatic interactions explained 15.9% of the phenotypic variation for grain yield in the doubled haploid population. One of these loci, on linkage group N19 had already shown a significant main effect as well. Three additional digenic epistatic interactions between 5 loci were identified at $P = 0.05$. Two of these loci exhibited significant additive effects and one of them (on N12) interacted with loci on two different linkage groups. One epistatic effect was negative, indicating that a recombinant allele combination increased grain yield. The rest of the effects were positive meaning that parental allele complexes contributed for higher grain yield. Thirteen loci involved in 7 epistatic interactions were identified in the testcross hybrids, which explained 33.4% of the phenotypic variation. Only one of these loci, on linkage group N3 showed a significant main effect. With midparent heterosis data were detected 18 loci in 9 pair wise interactions, explaining 36.6% of the phenotypic variance. None of these represent loci with significant main effects.

Thousand kernel weight: Twelve loci involved in 8 digenic interacions were detected in the doubled haploid population (Table 19). They explained 20.9% of the phenotypic variation. Two loci showed significant main effects with the locus on linkage group N7, beeing involved in two different epistatic interactions with opposite effects, showing that in one case (N7-N6) the parental allele combination contributed beneficially to seed weight, while in the other case (N7-N5) the recombinant allele combination increased seed weight. The QTL mapping in the testcross hybrid resulted in the detection of 12 loci in six combinations, which explained 28.4% of the phenotypic variation. One of these loci showed a significant main effect. Only 2 significant epistatic interactions were identified with midparent hetrosis data. They involved 4 loci and explained 11.9% of the phenotypic variance. The low number of epistatic interactions was in agreement with the low heterosis observed for thousand kernel weight (Table 15).

Seeds per silique: In total 16 loci involved in 8 digenic epistatic interactions, which explained 19.3% of the phenotypic variation, were detected in the doubled haploid population (Table 19). Seven showed positive effects, while one was negative. One of the loci involved in epistasis exhibited a significant additive effect as well. Two digenic interactions between four loci were identified in the testcross population. Together they explained 12.0% of the phenotypic variation of the trait. None of these loci was identical to a locus with main effect.

Epistatic interaction analyses with midparent heterosis data resulted in the identification of 23 loci involved in 14 epistatic interactions. With 51.8% the epistasis for seeds per silique explained a considerably higher portion of the phenotypic variance than the 4.3% explained by the main effect QTL. No loci with significant main effect were included in epistatic interactions.

Siliques per square decimetre: Fifteen loci in nine pair-wise combinations were mapped in the doubled haploid population, explaining 33.5% of the phenotypic variance. Four interactions were with negative and five with positive effects. One locus, on linkage group N19 showed also significant main effect. Only one interaction between two loci on the same linkage group, N18, that were 32.7 cM apart was identified in the testcross hybrids. The phenotypic variation explained was 8.0%. The analyses with midparent heterosis data led to the detection of 4 loci, involved in 2 epistatic interactions, explaining 10.5% of the phenotypic variation. The identification of only a small number of epistatic interactions was expected since no significant heterosis for siliques per square decimetre had been observed (Table 15).

Table 19 Epistatic interactions detected in the doubled haploid (DH) and testcross populations (TC) and the midparent heterosis values (MPH)

Trait[a]	Set[b]	LG	Pos[c]	LG	Pos	LOD	A_i[d]	A_j	AA_{ij}	$Vp_{(AAij)}$[e]
Y	DH	N2	169.0	N7	30.0	5.1			1.425***	6.46
Y	DH	N3	65.3	N12	34.2	13.5		1.85***	0.759*	0.00
Y	DH	N5	103.4	N6	36.7	6.1	-1.08***		0.725*	0.00
Y	DH	N11	9.5	N12	34.2	8.4		1.74***	0.540*	0.00
Y	DH	N13	29	N14	106.1	4.4			-1.215***	5.20
Y	DH	N14	40	N19	0.0	5.8		0.99***	0.964***	3.27
Y	TC	N2	19.8	N8	26.2	3.1			0.448***	4.76
Y	TC	N2	115.3	N3	15.0	4.0		0.62**	-0.348**	2.87
Y	TC	N3	112.1	N13	45.0	3.3			-0.427***	4.32
Y	TC	N13	25.0	N18	41.9	3.0			-0.413**	4.05
Y	TC	N13	54.9	N16	26.4	3.5			-0.463***	5.08
Y	TC	N15	59.4	N19	53.1	4.1			0.470***	5.24
Y	TC	N15	74.4	N16	30.4	4.9			-0.545***	7.05

For abbreviations see page 64

Table 19/Continued from page 61

Trait[a]	**Set**[b]	**LG**	**Pos**[c]	**LG**	**Pos**	**LOD**	**A_i**[d]	**A_j**	**AA_{ij}**	**$Vp_{(AAij)}$**[e]
Y	MPH	N2	0.0	N2	168.5	3.3			-0.586***	3.91
Y	MPH	N2	117.3	N3	0.0	6.5			-0.771***	6.76
Y	MPH	N3	57.3	N10	47.6	3.3			0.591***	3.97
Y	MPH	N3	106.1	N10	14.0	3.5			0.501***	2.86
Y	MPH	N9	8.1	N20	30.0	5.4			-0.689***	5.40
Y	MPH	N11	4.0	N15	57.6	4.8			-0.668***	5.08
Y	MPH	N11	69.6	N18	59.4	2.1			-0.423**	2.04
Y	MPH	N14	42.0	N19	0.0	4.9			-0.608***	4.21
Y	MPH	N14	128.1	N16	92.6	2.6			0.459**	2.40
TKW	DH	N1	33.3	N2	152.5	7.9			0.088***	4.98
TKW	DH	N4	96.1	N12	27.0	8.5		-0.08***	0.058***	2.16
TKW	DH	N4	96.1	N12	22.6	3.4			0.067**	2.89
TKW	DH	N5	151.0	N7	17.0	9.4		-0.10***	-0.063***	2.55
TKW	DH	N6	49.2	N14	50.0	3.3			-0.059***	2.24
TKW	DH	N6	55.2	N7	15.0	13.5		-0.11***	0.048**	1.48
TKW	DH	N6	82.8	N8	20.4	4.0			0.062***	2.47
TKW	DH	N13	47.0	N14	40.0	3.3			0.058***	2.16
TKW	TC	N1	114.2	N14	0.0	6.3	-0.05***		0.021**	2.05
TKW	TC	N3	8.0	N16	26.4	5.9			-0.04***	7.44
TKW	TC	N4	32.9	N5	34.2	4.6			0.036***	6.03
TKW	TC	N5	153.0	N15	59.4	5.2			0.032***	4.76
TKW	TC	N8	36.4	N16	71.9	3.7			-0.03***	4.19
TKW	TC	N15	127.9	N16	0.0	4.6			-0.029***	3.91
TKW	MPH	N8	28.2	N20	28.0	5.3			0.035***	7.64
TKW	MPH	N11	61.1	N15	29.7	4.1			-0.026***	4.21

For abbreviations see page 64

Table 19/Continued from page 62

Trait[a]	**Set**[b]	**LG**	**Pos**[c]	**LG**	**Pos**	**LOD**	**A_i**[d]	**A_j**	**AA_{ij}**	**$Vp_{(AAij)}$**[e]
S/Sil	DH	N1	72.8	N6	49.0	5.8			0.885***	3.60
S/Sil	DH	N1	98.2	N7	15.0	8.4			0.829***	3.16
S/Sil	DH	N3	0.0	N3	112.1	4.0			0.675***	2.09
S/Sil	DH	N5	78.8	N18	23.2	3.8			-0.609***	1.70
S/Sil	DH	N7	0.0	N17	41.4	3.9			0.627**	1.81
S/Sil	DH	N8	55.0	N16	89.3	3.3			0.613**	1.73
S/Sil	DH	N11	7.3	N12	12.2	12.0	-1.41***		0.784***	2.82
S/Sil	DH	N11	61.1	N17	58.1	4.4			0.722***	2.39
S/Sil	TC	N2	141.3	N4	18.0	4.8			-0.555***	6.21
S/Sil	TC	N15	87.2	N19	22.2	3.3			-0.535***	5.77
S/Sil	MPH	N2	148.7	N4	18.0	2.9			-0.417***	2.65
S/Sil	MPH	N2	152.5	N18	61.4	3.1			-0.397**	2.41
S/Sil	MPH	N3	6.0	N11	69.6	4.6			0.387**	2.29
S/Sil	MPH	N3	33.0	N9	19.0	6.9			-0.610***	5.68
S/Sil	MPH	N4	0.0	N19	52.0	4.0			-0.436**	2.90
S/Sil	MPH	N4	34.9	N13	13.5	3.9			-0.566***	4.89
S/Sil	MPH	N4	32.9	N18	26.5	4.8			0.622***	5.90
S/Sil	MPH	N5	2.2	N9	34.7	3.6			-0.494***	3.72
S/Sil	MPH	N5	167.0	N17	30.7	4.5			-0.634***	6.13
S/Sil	MPH	N9	8.1	N11	61.1	4.0			0.429**	2.81
S/Sil	MPH	N10	90.1	N17	24.7	4.7			-0.524***	4.19
S/Sil	MPH	N11	9.5	N15	125.9	3.8			-0.469***	3.36
S/Sil	MPH	N12	34.2	N18	91.6	2.7			0.402**	2.47
S/Sil	MPH	N13	31.3	N19	91.1	3.0			-0.398**	2.42
Sil/dm^2	DH	N2	2.0	N8	32.2	3.2			1.676***	3.69
Sil/dm^2	DH	N2	152.5	N5	107.4	3.9			-1.396***	2.56
Sil/dm^2	DH	N5	169.0	N7	17.0	3.8			-1.691***	3.75
Sil/dm^2	DH	N6	49.0	N10	57.6	4.4			1.352***	2.40
Sil/dm^2	DH	N8	36.4	N19	58.5	4.2		-1.50**	-1.823***	4.36
Sil/dm^2	DH	N11	41.4	N15	72.4	5.0			1.823***	4.36

For abbreviations see page 64

Table 19/Continued from page 63

Trait[a]	Set[b]	LG	Pos[c]	LG	Pos	LOD	A_i[d]	A_j	AA_{ij}	$Vp_{(AAij)}$[e]
Sil/dm^2	DH	N11	69.6	N19	26.2	1.9			1.074**	1.51
Sil/dm^2	DH	N14	36.1	N19	55.1	4.9			1.823***	4.36
Sil/dm^2	DH	N18	4.0	N18	26.5	4.9			-2.232***	6.54
Sil/dm^2	TC	N18	58.9	N18	91.6	3.4			1.255***	8.02
Sil/dm^2	MPH	N2	0.0	N20	4.0	3.2			-1.268**	5.86
Sil/dm^2	MPH	N4	0.0	N19	53.1	2.6			1.129**	4.65

[a] GY, TKW, S/Sil, Sil/dm^2: Grain yield [dt/ha], thousand kernel weight [g], seeds per silique and siliques per dm^2, respectively
[b]DH, TC, MPH: doubled haploid population, testcross hybrid population, and midparent heterosis dataset, respectively
[c]Positions are measured from the beginning of the linkage group in cM
[d]A_i, A_j: main effects at loci i and j; AA_{ij}: epistatic interaction effect between loci i and j
* Significant at $P = 0.05$; ** Significant at $P = 0.005$; *** significant at $P = 0.001$;
[e]Vp: Explained phenotypic variance [%]

3.6 Analysis of Plant Height and Phenological Traits

3.6.1 Analysis of Variance and Heritability

In addition to yield and yield related traits, also plant height and the phenological traits beginning, end and duration of flowering were evaluated in the field trials. The genetic variance and the heritability are presented in Table 20. Highly significant variation was observed for all traits under study in the doubled haploid, and testcross hybrid population and in the midparent heterosis data. A relatively low heritability was observed for plant height because the analysis was based on data from only two locations, due to scoring problems in Grund-Schwalheim and Rauischholzhausen. Similar to the heritability of the previously described traits, the heritability in the testcross hybrid population was lower than in the doubled haploid population.

Table 20 Genetic variance, effective error means and heritability of the doubled haploid lines, the test cross hybrids and the midparent heterosis data for plant height and the phenological traits

Population/Dataset Trait[a]	$\hat{\sigma}_g^2$	$\hat{\sigma}_e^2$	$\hat{h}^2$
DH lines			
PH	76.42**	47.65	0.76
BF	5.07**	1.08	0.93
EF	14.31**	5.80	0.88
DF	9.58**	6.05	0.83
TC hybrids			
PH	8.96**	26.39	0.40
BF	0.57**	0.38	0.82
EF	1.93**	3.05	0.66
DF	1.14**	2.99	0.53
MPH data			
PH	30.15**	98.52	0.55
BF	3.98**	1.17	0.91
EF	11.04**	7.76	0.81
DF	7.27**	8.10	0.73

[a]PH, BF, EF, DF: plant height , beginning of flowering, end of flowering, duration of flowering, respectively; $\hat{\sigma}_g^2$ genetic variance; $\hat{\sigma}_e^2$ effective error mean variance; $\hat{h}^2$ heritability

**Significant at $P = 0.01$

3.6.2 Correlations between Plant Height, Beginning, End, and Duration of Flowering

The correlations between plant height, beginning, end, and duration of flowering are presented in Table 21. Although the magnitudes of the correlations estimated in the doubled haploid, and testcross populations and in the midparent heterosis data differed, the signs of the significant correlation coefficients were always the same. Positive correlation was observed between plant height and the flowering related traits. Prolonged duration of flowering was determined predominantly by a later end of flowering and not by an earlier beginning of flowering.

Table 21 Correlations between plant height (PH) and beginning (BF), end (EF), and duration of flowering (DF) estimated in the doubled haploid lines, the test cross hybrids and the midparent heterosis data

Dataset/Trait	PH	BF	EF
DH-line population			
BF	0.389**		
EF	0.437**	0.518**	
DF	0.242**	-0.081	0.811**
TC-hybrid population			
BF	0.130*		
EF	0.279**	0.442**	
DF	0.240**	-0.050	0.874**
MPH data			
BF	-0.096		
EF	0.114	0.408**	
DF	0.184**	-0.130*	0.852**

*, **Significant at $P = 0.05$, $P = 0.01$, respectively. See Table 20 for abbreviations

3.6.3 Analysis of Heterosis for Plant Height, Beginning, End, and Duration of Flowering

F_1 heterosis and the performance of the F_1 hybrid, its parents and their midparent value are presented in Table 22. Table 23 shows the average testcross heterosis together with the performance of 'Express', the mean performance of the doubled haploid lines and the testcross hybrids, as well as the average midparent value of the doubled haploid lines with the tester 'MSL-Express'.

Table 22 F_1 and parental performance, midparent value and F_1 heterosis

Trait[a]					Heterosis[b]	
	Express ♀	R53 ♂	MPV	F_1	MPH	HPH
PH	143.72	150.22	146.97	170.64	16.0**	14.0**
BF	254.54	258.35	256.45	254.98	-1.5**	-3.7^{ns}
EF	279.64	284.68	282.17	283.93	1.8*	-0.8^{ns}
DF	25.1	26.30	25.71	28.94	3.2**	2.6**

[a]PH, BF, EF, DF: plant height [cm] , beginning of flowering [days after sowing], end of flowering [days after sowing], duration of flowering [days between BF and EF], respectively
[b]MPH and HPH: midparent heterosis and high parent heterosis estimated in percentage for PH and in days for BF, EF, and DF. *, **Significant at $P = 0.05$, $P = 0.01$, respectively, [ns] – nonsignificant

Table 23 Performance of 'Express', the doubled haploid population and the corresponding test cross hybrids as well as the average test cross midparent and high parent heterosis

		Mean of				
					Heterosis[b]	
Trait[a]	Express ♀	DH-Lines ♂	MPV	TC	MPH	HPH
PH	143.72	155.90	149.81	169.99	14.0**	9.0**
BF	254.54	257.38	255.96	255.19	-0.8**	-2.4**
EF	279.65	285.71	282.68	283.25	0.6**	-2.5**
DF	25.11	28.33	26.72	28.06	1.3**	-0.4[ns]

[a]PH, BF, EF, DF: plant height [cm] , beginning of flowering [days after sowing], end of flowering [days after sowing], duration of flowering [days between BF and EF], respectively
[b]MPH and HPH: midparent heterosis and high parent heterosis estimated in percentage for PH and in days for BF, EF, and DF. **Significant at P =0.01, [ns] – nonsignificant

Significant F_1- and average testcross midparent heterosis was observed for all traits under study, although for beginning and end of flowering it was of a low magnitude. Generally, hybrids quite often have a shorter vegetative period, flower earlier and have a slightly earlier maturity (Diepenbrock and Becker 1995), which explains the negative heterosis for beginning of flowering, but the hybrids of this population exhibited prolonged duration of flowering and positive heterosis for end of flowering as well. Except for plant height, there was again a clear reduction (about 50%) in the average testcross heterosis compared to F_1 heterosis as previously observed for yield and yield components.

3.6.4 Transgressive Segregation of Plant Height and the Phenological Traits

Figure 9 shows the distribution of phenotypic means for plant height and flowering-related traits of the doubled haploid lines and their corresponding testcross hybrids. For the traits under study the genetic variance in the testcross population was lower than in the doubled haploid population, as previously observed for yield and yield components. In case of plant height, where the highest level of heterosis was observed, still 19 doubled haploid lines were higher than the F1 hybrid, and 173 (69.2%) exceeded the higher parent R53. Twenty nine lines started to flower earlier and 16 reached maturity before the earlier parent 'Express'. One hundred and eighty three lines (73.2%) flowered for a longer duration than R53, and 83 lines showed a longer flowering period than the F_1 hybrid, although a significant heterosis of 12.6% for duration of flowering was observed. The transgressive segregation detected for the flowering-related traits indicate that the alleles for early entering in a generative phase are distributed between the two parents.

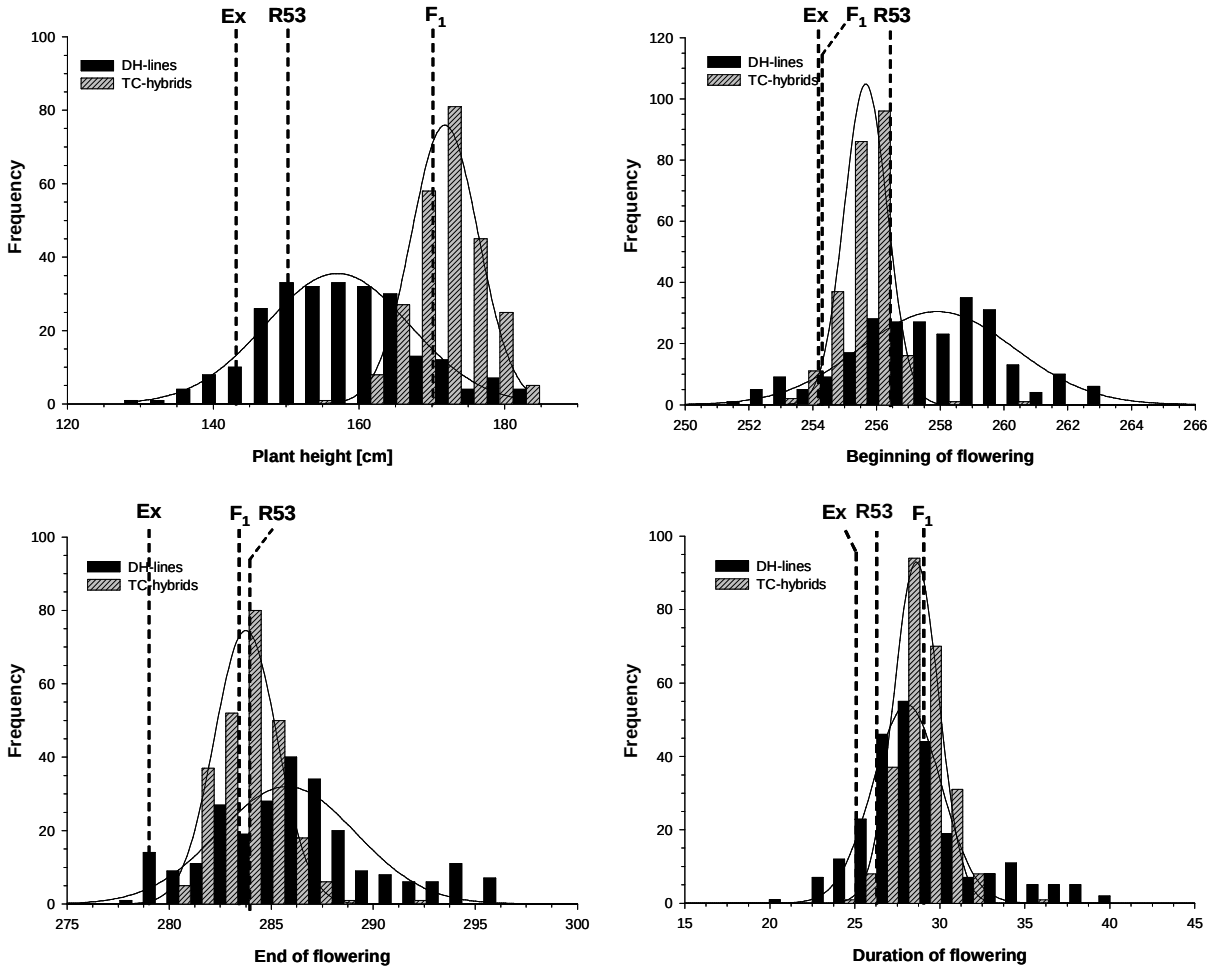


Fig. 9 Distribution of plant height, beginning, end, and duration of flowering in doubled haploid line and testcross hybrid populations. 'F_1', designate the mean value of the F1 hybrid, 'Ex' and 'R53' shows the mean values of the parents 'Express' and 'R53'.

3.6.5 Relationship of Plant Height and the Phenological Traits with Genome Heterozygosity

Similar to the traits described in previous sections, no significant correlation was observed between the overall genome heterozygosity and testcross hybrid performance (Table 24), pointing out that overall genome heterozygosity alone had little effect on trait expression in the testcross hybrid population**.**

Table 24 Correlation coefficients (r) and coefficients of determinations (R^2) between the genome heterozygosity and the trait value in testcross hybrid population (TC-hybrids) and midparent heterosis data (MPH)

Trait[a]	TC-hybrids		MPH	
	r	R^2	r	R^2
PH	0.082^{ns}	0.003	0.088^{ns}	0.004
BF	0.031^{ns}	-0.003	-0.134^{ns}	0.014
EF	-0.009^{ns}	-0.004	-0.040^{ns}	0.265
DF	-0.028^{ns}	-0.003	0.032^{ns}	-0.003

[a]PH, BF, EF, DF: plant height , beginning-, end-; and duration of flowering, respectively
ns – nonsignificant, **Significant at $P = 0.01$

3.6.6 Correlations between Line *per se*, Testcross Performance and Midparent Heterosis Values of Plant Height and Flowering-related Traits

The correlation analyses showed that different factors determined the testcross hybrid performance, as already described for yield and yield components. For beginning of flowering the variation of the testcross performance was determined predominantly by the variation of the mean performance of the doubled haploid parents instead of the variance in the heterosis (Table 25), which was consistent with the significant but very low level of heterosis observed for this trait (Table 23). For the rest of the traits, the correlation coefficients between the performance of the testcross hybrids, the doubled haploid lines, and the midparent heterosis were of similar magnitude, indicating that the variance of the performance of the parents and the levels of heterosis contribute to similar extent to the variation in the testcross hybrid performance. Negative correlations were observed between the doubled haploid line performance and the miparent heterosis for all traits under study.

Table 25 Phenotypic correlation between doubled haploid population (DH), testcross hybrid population (TC) and the midparent heterosis (MPH)

Trait[a]	Coefficient[b]	Between DH & TC	Between MPH & TC	Between DH & MPH
PH	r	0.413**	0.479**	-0.601**
BF	r	0.538**	0.150*	-0.754**
EF	r	0.464**	0.355**	-0.664**
DF	r	0.398**	0.429**	-0.658**

[a]PH, BF, EF, DF: plant height , beginning-, end-; and duration of flowering, respectively
[b]r – Pearson correlation coefficient, *,** Significant at $P = 0.05$, $P = 0.01$, respectively

3.6.7 QTL Mapping for Plant Height and the Phenological Traits

3.6.7.1 Analyses of Main Effect QTL

The main effect QTL analyses for plant height, beginning, end, and duration of flowering are summarised in Table 26 and Fig. 10.

Plant height: Six QTL significant at $P = 0.005$ were detected in doubled haploid population, which explained 27.7% of the phenotypic, and with a heritability of 0.76, 36.4% of the genotypic variation. Three QTL showed negative additive effects indicating that R53 contributed the alleles for higher plants. In the rest of the QTL these alleles came from 'Express'. A single QTL, with a rather large effect of 3.89 cm, explaining 7.8% of the phenotypic and 14.2% of the genotypic variance was identified with the midparent heterosis data on N12. No correspondent QTL with additive effect was detected at this locus, indicating overdominance. QTL mapping with testcross hybrid data resulted in the detection of 2 QTL, which explained 16.5% of the phenotypic and 41.3% of the genotypic variation.

Beginning of flowering: Nine QTL significant at $P = 0.005$ were mapped in the doubled haploid population. They explained 50.4% of the phenotypic and 54.2% of the genotypic variation. Two major QTL were identified on linkage groups N11 and N16, explaining 24.3% of the phenotypic variance. In 5 QTL, including the major ones, the additive effect was negative, showing that 'Express' contributed alleles for an earlier flowering time. In the remaining 4 QTL earlier flowering time was determined by alleles coming from the resynthesized parent. The QTL mapping with midparent heterosis data resulted in the detection of 3 QTL with dominance effects at $P = 0.005$, which explained 15% of the phenotypic and 16.5% of the genetic variance. An additional putative QTL at $P = 0.05$, congruent with a QTL with additive effect, was identified on linkage group N16. In all cases except the QTL on N19, the dominance effect was negative, explaining the negative heterosis for beginning of flowering. The dominance effect at the loci on linkage groups N5, N9, and N19 was indirectly calculated from the effects detected with the other two datasets. Six QTL were identified in testcross population, explaining 22.7% and 27.7% of the phenotypic and genotypic variation, respectively. In all cases except the QTL on N16, they overlapped with QTL showing additive or additive and dominance effects, allowing an estimation of the dominance effects at these positions even if the latter was under the detection threshold. A single QTL on linkage group N9 exhibited overdominance with a dominance ratio of 1.5. The remaining QTL where dominance effects could be calculated showed partial to full dominance with dominance ratio ranging from 0.1 to 0.9 (Table 26).

End of flowering: Nine QTL were mapped in doubled haploid population, which explained 34.5% of the phenotypic and 39.2% of the genotypic variance. The additive effects of 5 QTL were negative, indicating that 'Express' contributed alleles for a shorter flowering period. For the remaining 4 QTL, the alleles for earlier end of flowering were contributed by R53. Three QTL were identified with the midparent heterosis data, explaining 17.6% and 21.7% of the phenotypic and genotypic variation, respectively. The negative dominance effect of 2 of these QTL indicated that these QTL in heterozygous state would reduce flowering time with respect to the midparent value, which contradicted the positive heterosis observed for this trait. Three QTL were identified in the testcross population, explaining 21% of the phenotypic and 31.8% of the genotypic variance of the trait. None of these loci coincided with QTL showing additive and/or dominance effect. All QTL detected with dominance effects exhibited overdominance.

Duration of flowering: Five QTL at $P = 0.005$, were mapped in the doubled haploid data. They explained 22% of the phenotypic and 26.5% of the genotypic variance. The negative additive effect for 3 of the QTL, showed that the alleles of R53 determined a prolonged flowering period. The QTL mapping with the midparent heterosis data resulted in the detection of two QTL with dominance effects, which explained 10.5% and 14.4% of the phenotypic and genotypic variance, respectively. The QTL on linkage group N10 showed partial dominance with dominance ratio of 0.4, while the QTL on N11 exhibited overdominance (d/a = 1.2). No QTL were identified with the testcross hybrid data.

3.6.7.2 Analysis of Epistatic Interactions

The results of QTL analyses for epistasis are summarised in Table 27.

Plant height: Only a single interaction was identified in the doubled haploid population, between a QTL with a significant main effect and a locus without detectable main effect. The epistasis explained 3.3% of the variation for plant height in the doubled haploid population. Six loci involved in 3 digenic interactions were detected in the testcross population, explaining 17.9% of the phenotypic variance. One of the 6 loci exhibited a significant main effect as well. QTL mapping for epistasis with the midparent heterosis data resulted in the detection of 9 loci involved in 5 pair-wise combinations. One locus on linkage group N12 showed also a main effect. The epistatic interactions explained 39.3% of the phenotypic variance.

Table 26 QTL and their main effects detected in the doubled haploid line population (DH-Lines), the midparent heterosis values (MPH) and the testcross hybrid population (TC-Hybrids)

			DH-Lines				MPH				TC-Hybrids				
Trait[a]	**LG**	**Interval**	**Pos**[b]	**LOD**	**Effect**[c]	**Vp**[d]	**Pos**[b]	**LOD**	**Effect**[c]	**Vp**[d]	**Pos**[b]	**LOD**	**Effect**[c]	**Vp**[d]	**d/a**[e]
PH	N5	E35M62_249E - E32M47_41R	169.0	5.0	2.70***	6.0									
PH	N8	E35M62_128R - E32M48_296R	47.0	2.5	-2.11***	3.6									
PH	N11	Ol10E12 - E32M49_285R									12.1	6.3	-2.95***	9.3	
PH	N12	CB10600 - E35M62_117E					29.0	10.0	3.89***	7.8					
PH	N15	Na10G08b - Ol10B02	23.7	5.2	2.29***	4.3									
PH	N15	E32M48_82E - Na10G08b	12.0	3.9	2.36***	4.6									
PH	N16	BRAS048 - CB10211b	0.0	5.0	-2.59***	5.5									
PH	N16	CB10632 - CB10213									39.9	5.1	-2.59***	7.2	
PH	N16	CB10632 - CB10213	51.9	4.6	-2.13**	3.7									
BF	N5	E35M62_260R - MR119	63.2	3.3	0.40***	2.6			(±0.10)		64.6	4.1	0.30***	3.2	0.3
BF	N9	CB10022b - CB10311	96.6	7.9	-0.56***	5.0			(±0.29)		107.4	5.9	-0.27**	2.6	0.5
BF	N9	CB10092a - MR153d	22.7	7.9	-0.58***	5.4	20.7	13.1	-0.86***	8.1					1.5
BF	N10	CB10109b - Na10D07	23.4	4.1	0.41***	2.7									
BF	N11	CB10536 - CB10357b	2.0	16.1	-0.92***	13.6	0.0	9.2	-0.54***	5.0	0.0	3.5	-0.38***	5.3	0.6

For abbreviations see page 74

Table 26/Continued from page 72

Trait[a]	LG	Interval	DH-Lines				MPH				TC-Hybrids				d/a[e]
			Pos[b]	LOD	Effect[c]	Vp[d]	Pos[b]	LOD	Effect[c]	Vp[d]	Pos[b]	LOD	Effect[c]	Vp[d]	
BF	N15	CB10611 - E32M49_494R	57.6	9.0	-0.56***	5.0									
BF	N16	CB10213 - E32M47_152R	63.9	17.6	-0.82***	10.7	59.9	1.9	-0.27*	0.0	63.9	8.0	-0.25**	2.3	0.3
BF	N16	CB10632 - CB10213									47.9	7.6	-0.30***	3.2	
BF	N19	Ol10C10 - Na12E04a	52.0	4.4	0.46***	3.3			(±0.06)		52.0	4.5	0.40***	6.1	0.1
BF	N19	E32M49_400E - CB10357a	0.0	3.7	0.36**	2.1	12.2	4.2	0.33***	1.9					0.9
EF	N1	E32M49_51R - CB10597	87.9	2.2	-0.53**	1.5									
EF	N1	CB10597 - E32M51_340R									100.2	4.2	-0.68***	5.4	
EF	N8	E35M62_128R - E32M48_296R					55.0	3.8	-0.68***	2.8					
EF	N9	CB10373b - CB10022b	95.3	7.4	-0.96***	5.5									
EF	N10	Na10D07 - CB10552	33.6	9.6	-1.27***	7.8									
EF	N11	CB10005 - E32M48_273E	69.6	3.2	0.59**	1.7	65.6	7.3	0.88***	4.5					1.5
EF	N12	CB10600 - E35M62_117E	29.0	4.1	0.73**	2.6									
EF	N13	E32M47_113E - E32M51_161E	133.4	5.1	0.74***	2.6									
EF	N16	CB10632 - CB10213	47.9	3.7	-0.84***	3.4									
EF	N17	BRAS014 - MD50	62.1	7.7	-1.13***	6.2	49.7	8.9	-1.29***	10.3					1.1
EF	N19	CB10357a - Ol10D08	12.2	5.8	0.80***	3.2									
EF	N19	Ol10D08 - Ol10C10									35.5	7.2	0.70***	6.7	
EF	N19	CB10109a - CB10288									85.7	4.3	-0.88***	8.9	

For abbreviations see page 74

Table 26/Continued from page 73

Trait[a]	LG	Interval	DH-Lines				MPH				TC-Hybrids				d/a[e]
			Pos[b]	LOD	Effect[c]	Vp[d]	Pos[b]	LOD	Effect[c]	Vp[d]	Pos[b]	LOD	Effect[c]	Vp[d]	
DF	N1	E32M49_51R - CB10597	87.9	4.7	-0.77***	3.5									
DF	N10	CB10109b - Na10D07	29.4	16.1	-1.30***	9.9	27.4	3.2	-0.58***	2.8					0.4
DF	N11	MD60a - Ol10E12	9.5	6.4	0.82***	3.9	7.3	9.2	0.96***	7.7					1.2
DF	N12	CB10600 - E35M62_117E	29.0	3.2	0.62**	2.3									
DF	N13	E32M47_113E - E32M51_161E	141.4	6.1	0.75***	2.4									

[a]PH, BF, EF, DF: plant height [cm] , beginning of flowering [days after sowing], end of flowering [days after sowing], duration of flowering [days between BF and EF], respectively
[b]Positions are measured from the beginning of the linkage group in cM
[c]* Significant at $P = 0.05$; ** Significant at $P = 0.005$; *** Significant at $P = 0.001$;
The values in brackets are calculated from the effects at this locus detected with the other datasets
[d]Vp: Explained phenotypic variance [%]
[e]d/a: Dominance ratio

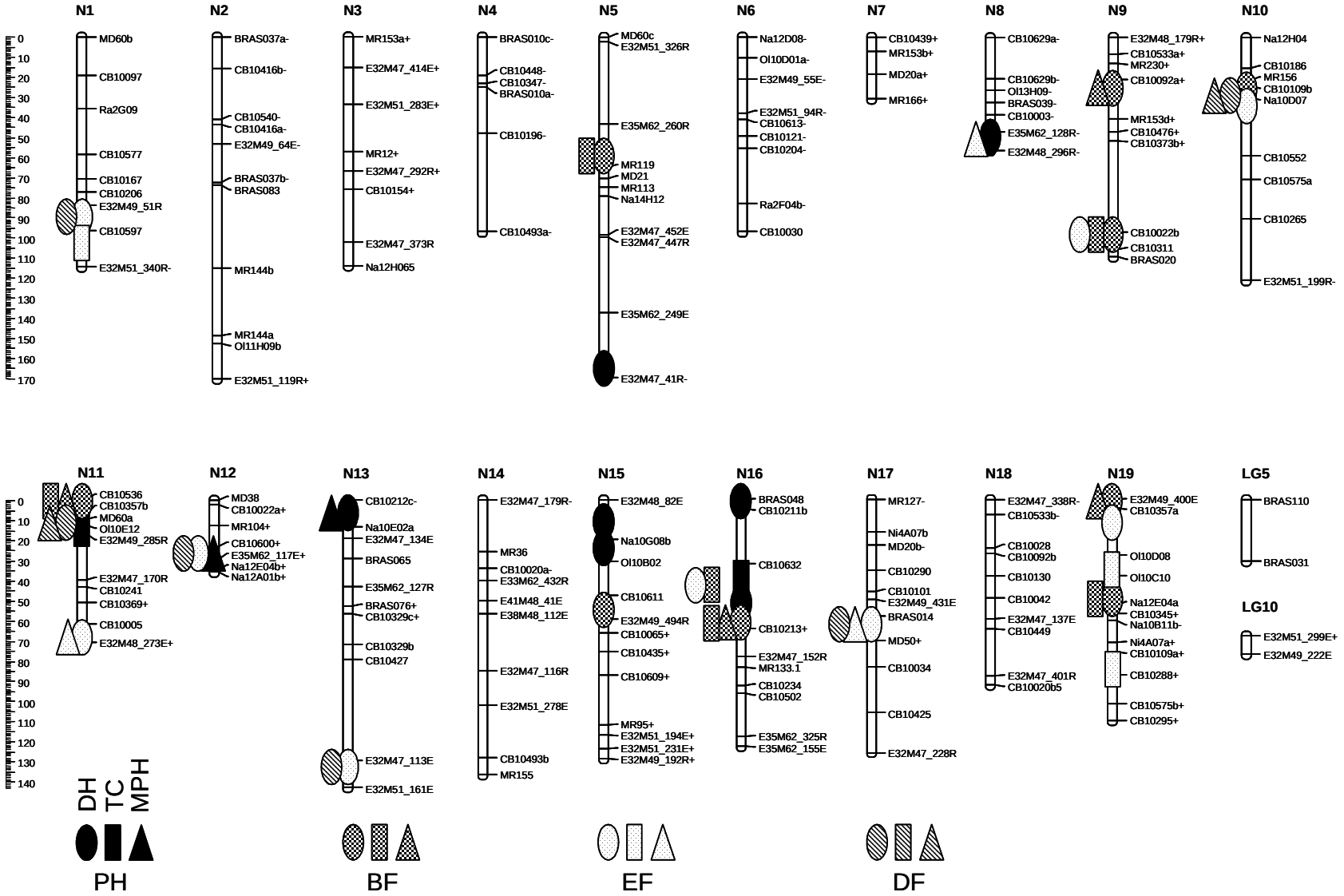


Fig. 10 Framework map of *B. napus* with QTL for plant height (PH), beginning (BF), end (EF), and duration (DF) of flowering. DH, TC and MPH are QTL detected with doubled haploid, and testcross hybrid population, and midparent heterosis data, respectively. For '+' and '-' signs at the end of the marker names see Fig 4. Positions are shown in cM estimated from the recombination frequencies by Haldanes mapping function.

Beginning of flowering: Four epistatic interactions, which explained 12.5% of the phenotypic variation for beginning of flowering, were identified in the doubled haploid population. They included 8 loci one of which showed a significant main effect as well. Three of the epistatic interactions were negative, while one additive x additive epistatic effect was positive. Eight loci involved in four digenic interactions were mapped with the testcross hybrid data. Two loci on linkage group N9 and N16 exhibited significant main effects as well. The phenotypic variance explained by epistasis was 20.2%. Fifteen loci involved in 8 digenic interactions were identified with the midparent heterosis data.They explained 27.7% of the phenotypic variance. One of the loci involved in epistatic interactions, on linkage group N11, had shown a significant main effect as well (Table 26, 27).

End of flowering: In total 11 loci involved in 6 digenic interactions were mapped in the doubled haploid population. Only one of these loci exhibited a significant main effect. Two interactions showed negative additive x additive epistatic effects, while for the remaining four interactions the epistatic effects were positive. The epistatic effects explained 19.2% of the phenotypic variation for end of flowering. The mapping in the testcross hybrid population led to the identification of 18 loci included in 9 pairs of loci. They explained 49.4% of the phenotypic variance of the trait. No locus with main effect was involved in epistatic interactions. Eighteen loci involved in 11 digenic interactions were localised with the midparent heterosis data. They explained 40.3% of the phenotypic variation, which was considerably higher than the 17.6% explained by the main effect QTL. For midparent heterosis, as well as in the testcross hybrid data, no QTL with significant main effect was involved in epistasis.

Duration of flowering: QTL mapping for epistatic interactions resulted in the detection of 12 loci involved in 7 pair-wise combinations in the doubled haploid population. Two of these loci showed significant main effect as well (Table 26, 27). The QTL on linkage group N10 was involved in two digenic interactions. In total 16.4% of the phenotypic variation for duration of flowering in the doubled haploid population was explained by epistasis. A large percentage of 54.7% of the phenotypic variation in the testcross population was explained by 14 digenic interactions including 24 loci. None of them showed significant main effects. No loci with significant main effects were identified in main effect QTL mapping as well, meaning that the whole variance explained for duration of flowering in the testcross population was due to epistasis. Twenty one loci involved in 11 digenic interactions were mapped with the midparent heterosis data. Only one of them showed a significant main effect.

In total the epistatic interactions explained 44.7% of the phenotypic variation, which was considerably higher than the 10.5% explained by main effects.

Table 27 Epistatic interactions detected in the doubled haploid (DH), and testcross (TC) populations and the midparent heterosis data (MPH)

Trait[a]	Set[b]	LG	Pos[c]	LG	Pos	LOD	A_i[d]	A_j	AA_{ij}	$Vp_{(AAij)}$[e]
PH	DH	N2	72.3	N15	23.7			2.29***	1.992***	3.25
PH	TC	N3	45.5	N11	12.1	6.3		-2.95***	1.256***	6.70
PH	TC	N9	20.7	N10	120.1	4.9			1.232***	6.45
PH	TC	N10	53.6	N17	12.0	3.5			-1.059***	4.77
PH	MPH	N1	64.4	N1	83.9	5.3			-2.221***	16.02
PH	MPH	N6	10.6	N14	56.7	3.6			1.153***	4.32
PH	MPH	N11	69.6	N16	71.9	4.0			1.287***	5.38
PH	MPH	N12	29.0	N16	71.9	10.0	3.1***		1.574***	8.04
PH	MPH	N13	15.5	N14	82.7	4.6			-1.306***	5.54
BF	DH	N1	0.0	N3	33.5	3.1			0.380***	2.31
BF	DH	N2	40.8	N15	111.9	6.0			-0.508***	4.13
BF	DH	N5	97.9	N12	29.0	4.1			-0.388***	2.41
BF	DH	N6	94.8	N15	57.6	9.0		-0.56***	-0.454***	3.30
BF	TC	N2	55.0	N14	84.9	4.6			-0.194***	5.42
BF	TC	N6	65.4	N16	47.9	7.6		-0.30***	0.206***	6.11
BF	TC	N9	107.4	N15	127.9	5.9	-0.27**		0.190***	5.20
BF	TC	N13	129.4	N14	130.4	3.3			0.155***	3.46
BF	MPH	N1	43.7	N8	30.2	3.8			-0.250***	4.30
BF	MPH	N2	40.8	N15	120.9	3.0			0.170***	1.99
BF	MPH	N2	69.0	N7	18.2	4.3			0.230***	3.64
BF	MPH	N2	115.3	N13	70.8	4.8			-0.189***	2.46
BF	MPH	N8	4.0	N16	75.9	11.5			-0.347***	8.29
BF	MPH	N10	25.4	N18	7.2	3.9			0.190***	2.49
BF	MPH	N10	29.4	N11	0.0	9.2		-0.54***	0.167***	1.92
BF	MPH	N12	12.6	N21	8.0	2.9			0.193**	2.57

For abbreviations see page 79

Table 27/Continued from page 77

Trait[a]	Set[b]	LG	Pos[c]	LG	Pos	LOD	A_i[d]	A_j	AA_{ij}	$Vp_{(AAij)}$[e]
EF	DH	N2	115.3	N9	12.1	4.7			-0.774***	2.88
EF	DH	N2	148.7	N19	101.1	6.3			0.836***	3.37
EF	DH	N6	20.7	N9	8.1	7.1			1.079***	5.61
EF	DH	N9	40.4	N10	33.6	9.6		-1.27***	0.827***	3.29
EF	DH	N11	12.1	N19	75.7	3.4			0.691***	2.30
EF	DH	N13	56.8	N19	87.1	3.4			-0.598**	1.72
EF	TC	N2	39.8	N12	2.0	3.7			0.330***	5.04
EF	TC	N2	101.5	N14	106.1	2.6			0.309**	4.42
EF	TC	N2	145.3	N6	47.0	3.3			-0.320***	4.74
EF	TC	N3	12.0	N16	121.9	2.6			0.294**	4.00
EF	TC	N3	57.3	N20	30.0	4.5			0.382***	6.75
EF	TC	N5	74.1	N5	99.4	3.9			-0.455**	9.58
EF	TC	N6	30.7	N16	30.4	2.8			0.284**	3.73
EF	TC	N11	16.3	N17	16.0	3.8			-0.316***	4.62
EF	TC	N16	102.4	N18	58.9	3.8			0.375***	6.51
EF	MPH	N1	72.8	N11	2.0	4.8			0.437***	4.75
EF	MPH	N1	106.2	N4	21.1	4.4			-0.409***	4.16
EF	MPH	N4	48.1	N13	13.5	5.0			0.435***	4.71
EF	MPH	N5	99.4	N10	90.1	5.0			-0.385***	3.69
EF	MPH	N10	15.0	N14	6.0	3.9			-0.409***	4.16
EF	MPH	N10	102.1	N18	47.9	3.6			0.384***	3.67
EF	MPH	N11	6.0	N15	6.0	3.2			-0.329***	2.69
EF	MPH	N12	0.0	N16	35.9	5.2			0.393***	3.84
EF	MPH	N13	74.0	N18	11.2	4.6			0.412***	4.22
EF	MPH	N14	0.0	N18	91.6	2.6			0.301**	2.25
EF	MPH	N15	75.6	N16	39.9	2.3			0.297**	2.19
DF	DH	N7	6.0	N15	65.4	4.6			0.543**	1.73
DF	DH	N9	51.1	N10	21.5	3.6			0.672***	2.64
DF	DH	N10	29.4	N11	0.0	16.1	-1.30***		-0.725***	3.08
DF	DH	N10	35.6	N15	65.4	15.6	-1.36***		-0.509**	1.52

For abbreviations see page 79

Table 27/Continued from page 78

Trait[a]	Set[b]	LG	Pos[c]	LG	Pos	LOD	A_i[d]	A_j	AA_{ij}	$Vp_{(AAij)}$[e]
DF	DH	N10	68.9	N17	101.3	3.4			0.566**	1.87
DF	DH	N11	14.1	N19	53.1	4.1			0.692***	2.80
DF	DH	N13	131.4	N17	22.7	7.7	0.65***		-0.682***	2.72
DF	TC	N3	21.0	N7	18.2	6.2			0.392***	7.20
DF	TC	N3	41.5	N20	30.0	3.9			0.311***	4.53
DF	TC	N4	96.1	N19	37.5	4.5			-0.303***	4.30
DF	TC	N6	10.0	N16	121.9	6.1			-0.332***	5.16
DF	TC	N6	12.6	N13	10.0	3.6			-0.238**	2.65
DF	TC	N6	39.9	N14	6.0	3.1			0.293***	4.02
DF	TC	N8	51.0	N19	37.5	3.1			-0.236**	2.61
DF	TC	N9	44.4	N15	2.0	3.2			-0.280***	3.67
DF	TC	N11	2.0	N17	16.0	3.0			-0.253***	3.00
DF	TC	N11	43.2	N16	78.0	4.0			0.232***	2.52
DF	TC	N12	0.0	N16	83.3	5.3			-0.322***	4.86
DF	TC	N13	29.3	N19	55.1	4.3			-0.295***	4.08
DF	TC	N13	47.0	N16	119.9	3.5			0.279***	3.65
DF	TC	N15	111.9	N16	69.9	3.5			0.228**	2.43
DF	MPH	N1	43.7	N14	27.6	3.0			-0.329***	3.62
DF	MPH	N1	74.8	N15	127.9	3.9			0.318***	3.39
DF	MPH	N1	79.3	N4	18.0	5.7			-0.375***	4.71
DF	MPH	N2	113.5	N5	72.1	3.0			-0.273**	2.50
DF	MPH	N4	48.1	N13	13.5	7.3			0.444***	6.60
DF	MPH	N5	97.9	N17	4.0	6.2			0.436***	6.37
DF	MPH	N6	20.7	N13	0.0	4.4			-0.366***	4.49
DF	MPH	N6	36.7	N16	30.4	3.2			0.270**	2.44
DF	MPH	N9	0.0	N10	2.0	4.7			0.358***	4.29
DF	MPH	N10	25.4	N13	141.4	7.4	-0.58***		0.341***	3.89
DF	MPH	N14	120.1	N17	124.1	2.2			0.267**	2.39

[a]PH, BF, EF, DF: plant height [cm] , beginning of flowering [days after sowing], end of flowering [days after sowing], duration of flowering [days between BF and EF], respectively
[b]DH, TC, MPH: doubled haploid population, testcross hybrid population, and midparent heterosis dataset, respectively
[c]Positions are measured from the beginning of the linkage group in cM
[d]A_i, A_j: main effects at loci i and j; AA_{ij}: epistatic interaction effect between loci i and j
* Significant at $P = 0.05$; ** Significant at $P = 0.005$; *** significant at $P = 0.001$;
[e]Vp: Explained phenotypic variance [%]

3.7 Analysis of Seed Quality Traits

3.7.1 Analysis of Variance and Heritability

After harvest the seed quality traits oil, protein, and glucosinolate content as well as erucic acid and sinapine content were analysed in the doubled haploid, the testcross populations, and with the midparent heterosis data. Significant genetic variation was observed for all quality traits studied in the three data sets (Table 28).

Table 28 Genetic variance, effective error mean and heritability of the doubled haploid lines test cross hybrids and midparent heterosis data

Population/Dataset Trait[a]	$\hat{\sigma}_g^2$	$\hat{\sigma}_e^2$	$\hat{h}^2$
DH lines			
Oil	2.31**	0.99	0.90
Pro	0.76**	0.70	0.81
GSL	192.51**	14.73	0.98
C22:1	81.42**	10.37	0.97
Sin	0.23**	0.34	0.73
TC hybrids			
Oil	0.34**	0.58	0.70
Pro	0.14**	0.40	0.58
GSL	27.13**	8.27	0.93
C22:1	14.57**	5.30	0.92
Sin	0.04**	0.22	0.41
MPH data			
Oil	1.32**	1.30	0.80
Pro	0.61**	0.95	0.72
GSL	105.34**	21.43	0.96
C22:1	40.10**	14.02	0.92
Sin	0.14**	0.55	0.51

[a]Oil, Pro, GSL, C22:1, Sin: oil, protein, glucosinolates, erucic acid, and sinapine, respectively

$\hat{\sigma}_g^2$ genetic variance; $\hat{\sigma}_e^2$ effective error mean variance; $\hat{h}^2$ heritability

**Significant at $P = 0.01$

With 0.98 and 0.97 the highest heritabilities were detected for the glucosinolate and erucic acid content, respectively, in the doubled haploid. These oligogenic traits are determined by a low number of genes, each of which explains a large portion of the genotypic variation and is not strongly influenced by environment, which explains the high heritabilities. The observed trend for lower heritabilities in the testcross hybrid population in comparison with the doubled haploid population, that have been observed for the yield related and the phenological traits, was still recognisable in the quality traits but not as pronounced, especially considering glucosinolate and erucic acid content, where the heritability dropped only slightly from 0.98 to 0.93 and from 0.97 to 0.92, respectively.

3.7.2 Correlations between Quality Traits

The correlations between the quality traits are presented in Table 29. For most trait combinations only the magnitude of the correlation coefficients changed in the different datasets but not the direction of the correlation, as it had already been seen for the previously studied traits.

Table 29 Correlations between oil, protein, glucosinolate, erucic acid, and sinapine content estimated for the doubled haploid lines, the test cross hybrids and the midparent heterosis data

Dataset/Trait	Oil	Pro	GLS	C22:1
DH-line population				
Pro	-0.514**			
GLS	-0.326**	0.339**		
C22:1	0.588**	-0.012	0.025	
Sin	-0.185**	-0.129*	-0.025	-0.436**
TC-hybrid population				
Pro	-0.584**			
GLS	-0.102	0.205**		
C22:1	0.589**	-0.030	0.105	
Sin	-0.287**	0.072	-0.098	-0.335**
MPH data				
Pro	-0.718**			
GLS	-0.341**	0.358**		
C22:1	0.355**	0.008	0.074	
Sin	-0.025	0.020	-0.022	-0.090

*, **Significant at $P = 0.05$, $P = 0.01$, respectively. See Table 28 for abbreviations

For the quality traits an exception from the rule was observed for the correlations between protein and erucic acid content and between protein and sinapine content but in both cases the correlation coefficients which changed their signs, in comparison with the doubled haploid population were not significant. The high positive correlation between oil and erucic acid content and the negative correlations observed between oil and protein and erucic acid and sinapine content is an indication that common genetic factors are responsible for the correlated traits, which was shown later at QTL level

3.7.3 Analysis of Heterosis for Quality Traits

Erucic acid and glucosinolate content, which are segregating in the plant material used in the current study, are oligogenic determined and are not expected to show much heterosis, which is a phenomenon predominantly observed in complex polygenic traits. This general expectation was, to great extent, in agreement with the results of the heterosis analysis for the quality traits shown in Tables 30 and 31. Deviations from the expectation were detected for oil and protein content. Very low in magnitude but statistically significant levels of averge testcross midparent heterosis were observed for oil and protein content but in opposite direction (Table 31). Protein content also displayed statistically significant F_1 midparent heterosis (Table 30). The lack of significant midparent heterosis for most of the quality traits is an indication for additivity.

Table 30 F_1 and parental performance, midparent value and F_1 heterosis

Trait[a]					Heterosis (%)[b]	
	Express ♀	R53 ♂	MPV	F_1	MPH	HPH
Oil	43.63	40.92	42.27	42.70	1.0^{ns}	-2.1**
Pro	19.59	22.90	21.24	20.65	-2.8**	-9.8**
GLS	14.47	36.12	25.29	22.75	-10.1^{ns}	-37.0**
C22:1	7.83	25.05	16.44	16.46	0.1^{ns}	-34.3**
Sin	7.71	7.68	7.69	7.71	0.2^{ns}	0.0^{ns}

[a]Oil, Pro, GLS, C22:1, Sin: oil [%], protein [%], glucosinolates [µmol/g], erucic acid [%] and sinapine, respectively
[b]MPH and HPH: midparent heterosis and high parent heterosis. **Significant at $P = 0.01$, ^{ns} – nonsignificant

Table 31 Performance of 'Express', the doubled haploid population and the corresponding test cross hybrids as well as the average test cross midparent and high parent heterosis

		Mean of				
					Heterosis (%)[b]	
Trait[a]	Express ♀	DH-Lines ♂	MPV	TC	MPH	HPH
Oil	43.63	41.29	42.46	42.78	0.8**	-2.1**
Pro	19.59	22.24	20.92	20.71	-1.0**	-6.8**
GLS	14.47	31.64	23.05	22.51	-2.3^{ns}	-21.2**
C22:1	7.83	22.43	15.13	15.83	8.7^{ns}	-20.0**
Sin	7.71	7.10	7.40	7.42	0.3^{ns}	-4.2**

[a]Oil, Pro, GLS, C22:1, Sin: oil [%], protein [%], glucosinolates [µmol/g], erucic acid [%] and sinapine, respectively
[b]MPH and HPH: midparent heterosis and high parent heterosis. **Significant at $P = 0.01$, ^{ns} – nonsignificant

3.7.4 Transgressive Segregation Observed in Quality Traits

The distribution of phenotypic means for seed quality traits of the doubled haploid lines and their corresponding testcross hybrids are presented in Fig. 11. The phenotypic distributions of oil, protein and sinapine for both doubled haploid lines and testcross hybrids approached normal distribution, indicating that these traits are determined by high number of genes, which are under environmental influence. The distributions of glucosinolate and erucic acid content, which are typical oligogenic traits, deviated considerably from the normal distribution (Fig 13). For most of the studied traits no significant heterosis was observed (Table 30 and 31), explaining why the F_1 hybrid value fell inbetween the two parents in the vicinity of the midparent value (Fig. 11). Pronounced transgressive segregation was observed for all traits under study. Eighteen doubled haploid lines exceeded the oil content of 'Express', which was the better parent for this trait. Sixty two doubled haploid lines showed higher protein level than R53, but not a single one had lower protein content than 'Express'. No significant difference for sinapine content was observed between the parents 'Express' and 'R53', but the 34 doubled haploid lines with higher sinpine levels, indicate that different alleles determined the trait in the conventional cultivar and the resynthesized line. A large number of lines 105 out of 250 exceeded 'R53' – the parent with higher glucosinolate content, while 19 lines exhibited lower values , than 'Express'. Unexpectedly an apparent transgressive segregation was observed for erucic acid content, as well where 146 lines outperformed the resynthesized parent 'R53'

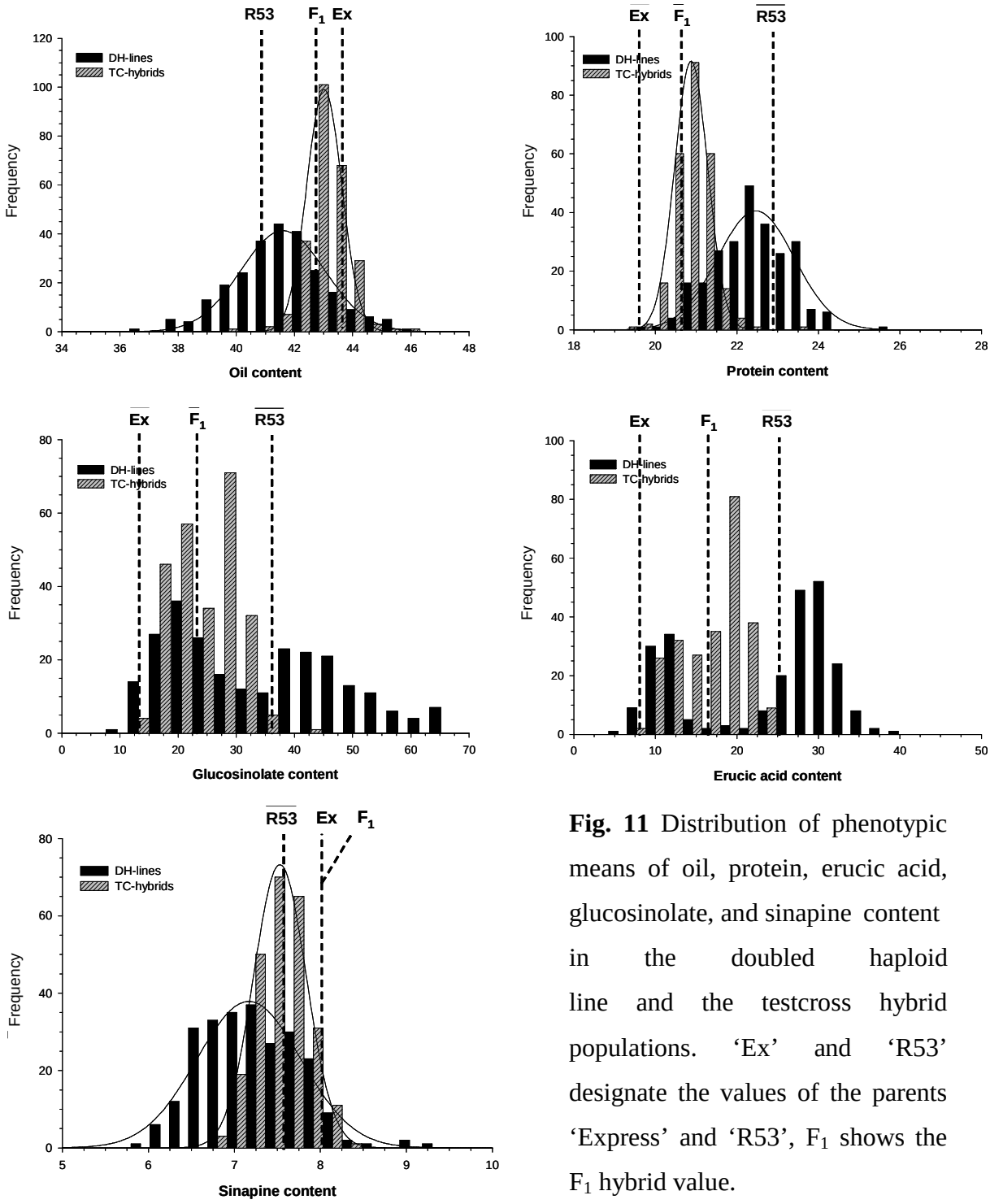


Fig. 11 Distribution of phenotypic means of oil, protein, erucic acid, glucosinolate, and sinapine content in the doubled haploid line and the testcross hybrid populations. 'Ex' and 'R53' designate the values of the parents 'Express' and 'R53', F_1 shows the F_1 hybrid value.

3.7.5 Quantitative Trait Loci Analyses for Seed Quality Traits

3.7.5.1 Analyses of Main Effect QTL

The results of main effect QTL mapping for seed quality traits using the three datasets (chapter 2.2.11.3) are presented in Table 32 and Fig. 12.

Oil content: Eight QTL significant at $P = 0.005$ were detected in the doubled haploid population. Together they explained 29.6% of the phenotypic and 32.8% of the genotypic variance. The increasing allele of the major QTL detected on linkage group N8, which alone explained 15.2% of the phenotypic variance, was contributed by the resynthesized parent and represents the QTL correspondent to one of the two well studied erucic acid genes. The allele for higher oil content of the QTL on N11 was contributed by 'R53' as well. 'Express' contributed the beneficial alleles for the remaining 6 QTL. Three QTL were localised with the midparent heterosis data, which explained 16.0% of the phenotypic and 20.0% of the genetic variance. All of them showed positive dominance effects, indicating that the allele increasing the trait was dominant. Two of them on linkage group N5 and N19 showed full dominance with dominance ratios of 0.94 and 1.00, respectively. The QTL on N14 was detected only with the midparent heterosis data, which is an indication for overdominance. The dominance effects at two loci were calculated from the effects estimated with the other datasets (chapter 2.2.11.5 and Appendix 3). Of these, the QTL on N8 showed nearly additivity with a dominance ratio of only 0.08, while the other QTL exhibited low partial dominance with a dominance ratio of 0.38. The QTL mapping with testcross hybrid data resulted in the detection of three QTL, explaining 37.8% of the phenotypic and 54.0% of the genotypic variance. The positive effects of two of them indicated that the loci homozygous for 'Express' alleles contributed for higher oil content than the heterozygous state of these loci. In case of the third QTL, on N8, the testcross hybrids, which carried an allele for erucic acid from the resynthesized parent, showed higher oil content than 'Express'.

Protein content: Seven QTL were mapped with the doubled haploid population, explaining 31.2% of the phenotypic and 38.5% of the genotypic variance. Six of them were with negative additive effect showing that the resynthesized parent contributed the alleles determining higher protein content. Only the QTL on N5 showed positive effect. A single QTL was detected with midparent heterosis data, which explained 4.3% of the phenotypic and 5.9% of the genotypic variation. It exhibited negative overdominance. After a calculation of the dominance effect of the QTL on N5 (Appendix 3, chapter 2.2.11.5), a dominance ratio of 0.3 was assessed, indicating partial dominance. Three QTL were identified with the testcross

hybrid data, explaining 16.1%, and 27.8% of the phenotypic and genotypic variance respectively.

Glucosinolate content: Five QTL were detected in the doubled haploid population, which explained 26.0% of the phenotypic and 26.5% of the genotypic variance. The negative additive effect of three QTL showed that the resynthesized parent contributed the alleles for high glucosinolate content at these loci. For the other two QTL 'Express' alleles increased the glucosinolate content. The QTL on linkage group N19 was a major QTL alone explaining 23.5% and 24.0% of the phenotypic and genotypic variation, respectively. Besides the additive effect, this QTL displayed a negative dominance effect, as well, meaning that at this locus the allele decreasing glucosinolate content was dominant. The dominance ratio reached 0.3, indicating partial dominance. Two additional minor QTL with positive dominance effects were identified on linkage groups N11 and N16. Both of them exhibited overdominance. Together the QTL with dominance effect explained 31.0% of the phenotypic and 32.3% of the genotypic variance. The QTL mapping for glucosinolate content with the testcross hybrid data resulted in the detection of three QTL, which explained 24.3% of the phenotypic and 26.1% of the genotypic variation. Again, with 22.7% the major QTL on N19 explained the largest portion of the variation. All of the detected QTL were with negative effect indicating that the heterozygous state at these loci contributed to higher glucosinolate content than the homozygous state for 'Express' alleles.

Erucic acid content: A single major QTL, which alone explained 62.7% and 64.6% of the phenotypic and genotypic variation, respectively, was identified in the doubled haploid population. The negative sign of the additive effect indicated that the high erucic acid allele is contributed by the resynthesized parent. QTL at the same position were identified with the testcross hybrid and midparent heterosis data, explaining 52.9% and 18.3% of the phenotypic variance, respectively. The identified negative partial dominance (d/a = 0.3), showed that for this locus the allele decreasing the trait was dominant. An additional minor QTL, controlling erucic acid content was detected on linkage group N19. It explained 3.6% of the phenotypic variation. The positive additive effect indicated that, surprisingly, the increasing allele was contributed by 'Express', a variety of canola quality. This could be a result of earlier maturity of 'Express' providing better conditions for a synthesis of long fatty acids. On linkage group N14 a QTL was localised with the midparent heterosis data, which explained 8.3% of the phenotypic variance. Its positive dominance effect was of a similar magnitude as the negative dominance effect detected on linkage group N8. The opposite directions of the two observed dominance effects may be the reason for the lack of midparent heterosis on a population level.

Table 32 QTL and their main effects detected in the doubled haploid line population (DH-Lines), the midparent heterosis values (MPH) and the testcross hybrid population (TC-Hybrids)

			DH-Lines				MPH				TC-Hybrids				d/a[e]
Trait[a]	LG	Interval	Pos[b]	LOD	Effect[c]	Vp[d]	Pos	LOD	Effect[c]	Vp	Pos	LOD	Effect[c]	Vp	
Oil	N5	E32M51_326R - E35M62_260R	6.2	8.3	0.38***	2.8	14.2	6.8	0.36***	7.3					0.9
Oil	N8	BRAS039 - CB10003	34.4	27.7	-0.88***	15.2			(±0.07)		38.4	33.3	-0.81***	32.1	0.1
Oil	N9	CB10533a - MR230	12.0	11.7	0.22***	1.0									
Oil	N11	E32M49_285R - E32M47_170R	38.3	5.6	-0.27***	1.4									
Oil	N12	CB10600-E35M62_117E	25.0	5.4	0.32***	2.0			(±0.12)		29.6	7.0	0.20**	2.7	0.4
Oil	N13	CB10329c - CB10329b									64.8	8.0	0.25***	3.0	
Oil	N13	CB10329b - CB10427	76.0	10.0	0.45***	3.8									
Oil	N14	E33M62_432R - E41M48_41E					40.0	3.2	0.26**	3.9					
Oil	N19	CB10575b - CB10295	103.8	29.4	0.30***	1.8									
Oil	N19	Ol10D08 - Ol10C10	37.5	5.9	0.29***	1.6	33.5	4.4	0.29***	4.8					1.0
Pro	N1	CB10206 - E32M49_51R	79.3	4.0	-0.14**	2.0									
Pro	N1	CB10597 - E32M51_340R									114.2	3.2	-0.20***	4.6	
Pro	N5	E32M51_326R - E35M62_260R					16.2	5.6	-0.20***	4.3					
Pro	N5	E32M47_41R - E35M62_249E	99.4	9.6	0.30***	9.1			(±0.09)		99.4	4.4	0.21***	5.4	0.3
Pro	N7	MR153b - MD20a	7.0	6.5	-0.24***	5.9									
Pro	N7	MD20a - MR166									24.2	6.5	-0.23	6.1	

For abbreviations see page 89

Table 32/Continued from page 87

Trait[a]	LG	Interval	DH-Lines				MPH				TC-Hybrids				d/a[e]
			Pos[b]	LOD	Effect[c]	Vp[d]	Pos	LOD	Effect[c]	Vp	Pos	LOD	Effect[c]	Vp	
			8.1	5.6	-0.20***	3.8									
Pro	N10	CB10552 - CB10575a	68.9	4.0	-0.17***	2.8									
Pro	N10	CB10575a - CB10265	82.8	5.1	-0.16**	2.6									
Pro	N15	Ol10B02 - CB10611	46.6	15.2	-0.22***	5.0									
GLS	N1	MD60b - CB10097	0.0	5.0	1.76***	0.5									
GLS	N5	MD21 - MR113									74.1	4.8	-1.40***	0.6	
GLS	N7	CB10439-MR153b	2.0	6.6	-2.26***	0.8									
GLS	N7	MD20a - MR166									18.2	5.3	-1.79***	1.0	
GLS	N11	MD60a - Ol10E12	9.5	5.2	1.63***	0.4	14.1	12.0	2.57***	9.6					1.6
GLS	N13	CB10427 - E32M47_113E	79.5	6.3	-2.22***	0.8									
GLS	N16	CB10213 - E32M47_152R					65.9	6.7	1.85***	4.9					
GLS	N19	Ol10D08 - Ol10C10	29.5	80.2	-12.03***	23.5	29.5	15.7	-3.37***	16.5	27.5	51.3	-8.25***	22.7	0.3
C22:1	N8	BRAS039 - CB10003	34.4	64.6	-6.92***	62.7	32.4	20.0	-2.20***	18.3	34.4	53.0	-5.40***	52.9	0.3
C22:1	N14	E33M62_432R - E41M48_41E					44.0	8.6	1.48***	8.3					
C22:1	N19	CB10575b - CB10295	107.8	7.2	1.65***	3.6									

For abbreviations see page 89

Table 32/Continued from page 88

			DH-Lines				MPH				TC-Hybrids				d/a[e]
Trait[a]	**LG**	**Interval**	**Pos[b]**	**LOD**	**Effect[c]**	**Vp[d]**	**Pos**	**LOD**	**Effect[c]**	**Vp**	**Pos**	**LOD**	**Effect[c]**	**Vp**	
Sin	N1	Ra2G09 - CB10577	57.7	6.2	0.13***	2.4									
Sin	N3	MR12 - E32M47_292R	65.3	4.3	-0.10***	1.4									
Sin	N5	MD21 - MR113	72.1	8.0	-0.13***	2.4	74.1	3.9	-0.11**	2.7					0.8
Sin	N8	BRAS039 - CB10003	36.4	20.3	0.26***	10.0			(±0.10)		32.2	9.6	0.16***	7.0	0.4
Sin	N9	CB10092a - MR153d	24.7	12.9	-0.21***	6.4			(±0.10)		20.7	2.5	-0.11**	3.4	0.5
Sin	N10	Na12H04 - CB10186	4.0	6.8	-0.14***	3.0									
Sin	N10	MR156 - CB10109b									23.4	3.1	-0.12***	3.8	
Sin	N16	CB10211b - CB10632	4.4	3.0	-0.08***	1.0									
Sin	N17	BRAS014 - MD50	58.1	4.8	0.11***	1.9									
Sin	N18	CB10130 - CB10042	41.9	3.8	-0.12***	2.2									
Sin	N18	E32M47_137E - CB10449	61.4	8.4	0.14***	2.8									

[a] Oil, Pro, GLS, C22:1, Sin: oil [%], protein [%], glucosinolate [µmol/g], erucic acid [%], and sinapine [mg/g], respectively
[b] Positions are measured from the beginning of the linkage group in cM
[c] * Significant at $P = 0.05$; ** Significant at $P = 0.005$; *** Significant at $P = 0.001$;
The values in brackets are calculated from the effects at this locus detected with the other datasets
[d] Vp: Explained phenotypic variance [%]
[e] d/a: Dominance ratio

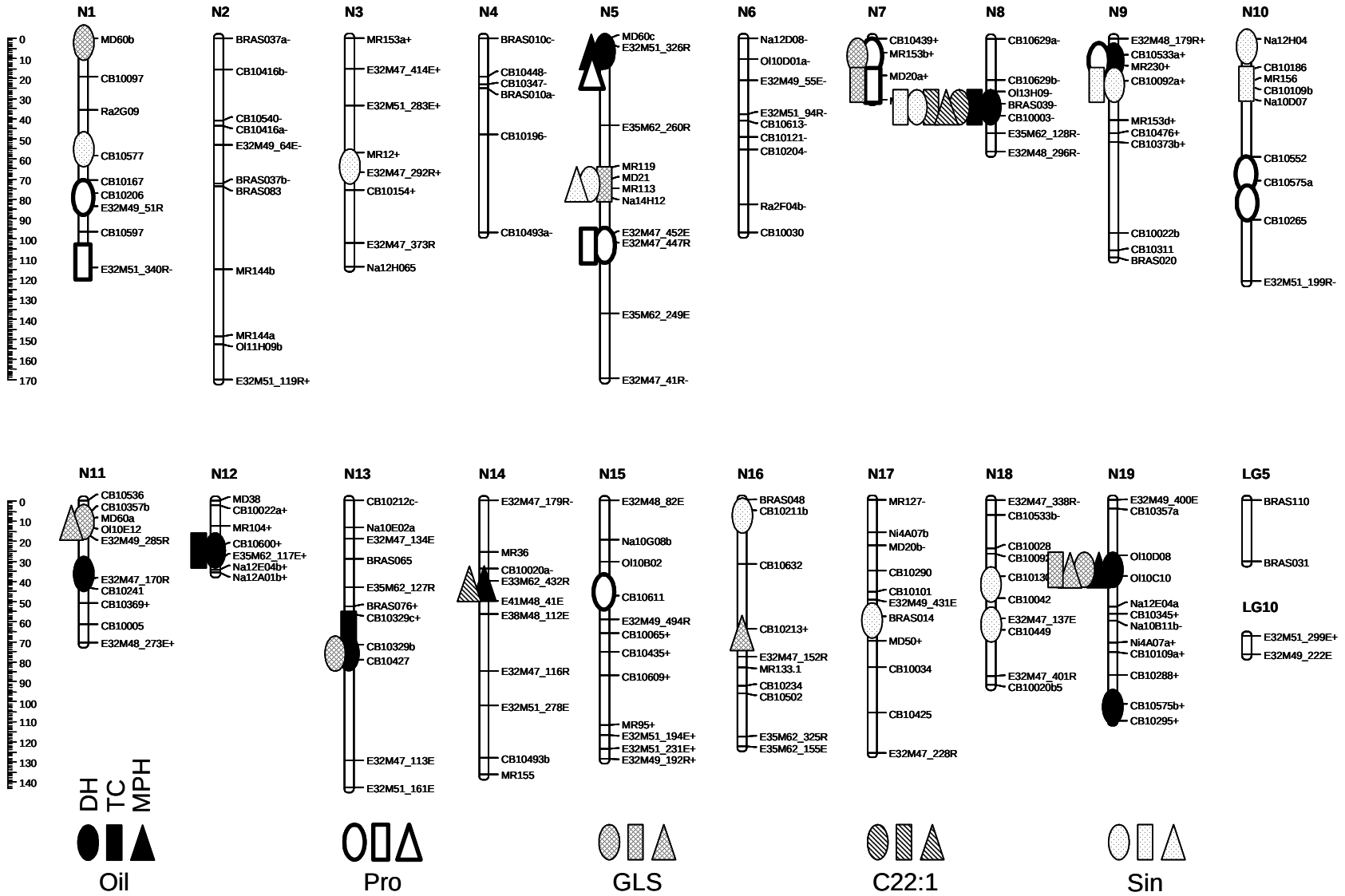


Fig. 12 Framework map of *B. napus* with QTL for Oil (Oil), Protein (Pro), Glucosinolate (GLS), Erucic acid (C22:1), and Sinapine (Sin). DH, TC and MPH are QTL detected in the doubled haploid population, the testcross population, and the midparent heterosis data, respectively. Marker positions are calculated from the recombination frequencies according to Haldanes mapping function. For '+' and '-' signs at the end of the marker names see Fig 4.

Sinapine content: A total number of 10 QTL, which explained 33.5% of the phenotypic and 45.9% of the genotypic variance were mapped in the doubled haploid population. For six of the QTL the alleles increasing the sinapine content were contributed by 'R53'. For the other four QTL 'Express' contributed the increasing alleles. The QTL on linkage group N8, had a major effect, explaining already 10% of the phenotypic variance. It coincided with the major QTL for erucic acid (Fig. 12), but showed an opposite sign of the additive effect, which is a hint for a pleiotropic effect of the gene in this locus. A single QTL, explaining 2.7% of the phenotypic variation was identified with midparent heterosis data. The dominance effect of two additional QTL was calculated according to Appendix 3 and chapter 2.2.11.5. In all cases partial dominance was observed with a dominance ratio of 0.8, 0.4, and 0.5 for the QTL on linkage groups N5, N8, and N9, respectively. Three QTL were identified with the testcross hybrid data, together explaining 14.2% of the phenotypic and 34.6% of the genotypic variance.

3.7.5.2 Analyses of Epistatic Interactions

The results of QTL analyses for epistasis are summarised in Table 33.

Oil content: In total 14 loci involved in 8 digenic epistatic interactions, explaining 10.5% of the phenotypic variation, were identified in the doubled haploid population. One of these loci, on linkage group N19, had already exhibited main effect significant at $P = 0.005$ (Tables 32, 33). Only one of the eight epistatic interactions was between a main effect QTL and a modifying locus, while the remaining interactions included background loci. Twenty loci involved in 12 pair-wise combinations were detected in the testcross population, explaining 29.4% of the phenotypic variance. Two of them, on linkage groups N12 and N13 had already been shown to display significant main effects (Tables 32, 33). The mapping with midparent heterosis data led to the detection of 6 loci involved in 3 digenic interactions, which explained 14.2% of the phenotypic variance. One pair of loci included a locus with a main effect, while the other interactions were between background loci.

Protein content: Seven digenic interactions between 14 loci, explaining 23.3% of the phenotypic variation, were identified in the doubled haploid population. One of these loci, on linkage group N10, showed a significant main effect (Tables 32, 33). With the testcross hybrid data six loci forming 3 pair-wise combinations could be mapped. Together they explained 15.7% of the phenotypic variation. Only one of the loci involved in epistatic interactions displayed a significant main effect. Nineteen loci included in 10 digenic combinations were identified with midparent heterosis data. Just a single locus showed a main

effect as well. In total the epistasis explained 39.9% of the phenotypic variation, which was considerably higher than the 4.3% explained by the main effect QTL.

Glucosinolate content: Epistatic QTL mapping for glucosinolate content in the doubled haploid population resulted in the detection of 15 loci, involved in 8 pair-wise combinations. Only 3.3% of the phenotypic variation was explained by epistasis. No main effect QTL was involved in interaction. Sixteen loci involved in 8 digenic epistatic interactions, explaining 6.7% of the phenotypic variance were identified with the testcross hybrid data. The interactions included only background loci. With the midparent heterosis data were localised 15 loci in 8 pair-wise combinations. Two loci, on linkage group N11 and N16, had already demonstrated significant main effects (Tables 32, 33). In total the epistasis explained 26.2% of the phenotypic variance, which was similar to the 31.0% explained by the main effect QTL.

Erucic acid content: Thirteen loci involved in 7 digenic epistatic interactions were detected in doubled haploid population. Both of the loci showing significant main effects were included in epistatic interactions. In all cases, except the interaction between the loci on N3 and N8, the epistatic effects were positive indicating that parental allele combination contributed for higher erucic acid content. Despite the considerably high number of loci involved in epistatic interactions, the phenotypic variance explained by them was only 11.6%, while 66.3% of the phenotypic variation was due to segregation of mainly one main effect QTL. In the testcross population 21 loci involved in 13 digenic interactions were identified. The single main effect QTL mapped here (Tables 32, 33) was involved in 4 pairs of epistatic interactions. In total the epistasis was responsible for 27.5% of the phenotypic variation, while 52.9% had been explained by the main effect of the QTL on N8. Thirteen digenic interactions between 23 loci were mapped with midparent heterosis data. None of the loci with main effects was involved in these interactions. Jointly they explained 39.3% of the phenotypic variance, which was slightly higher than the 26.6% of the phenotypic variance explained by the two main effect QTL (Table 32).

Sinapine content: Fifteen loci involved in nine digenic interactions, which explained 10.2% of the phenotypic variation, were identified with the doubled haploid population. Three of these loci exhibited significant main effect (Table 32, 33). Two of them interacted between each other, while the third one was involved in a combination with a modifying locus. The rest of the epistatic interactions were between background loci. Nine loci involved in five pair-wise combinations, explaining 23.7% of the phenotypic variation, were mapped in the testcross population. Only one of them, on linkage group N8, exhibited a main effect

(Tables 32, 33). The mapping with the midparent heterosis data resulted in the localization of 17 loci, forming 9 digenic combinations. Only background loci without significant main effects were involved in these combinations. The phenotypic variance explained by epistasis reached 41.6%, which was considerably higher than the 2.7% explained by the single main effect QTL identified with the midparent heterosis data.

Table 33 Epistatic interactions detected in the doubled haploid (DH), and the testcross (TC) populations and with the midparent heterosis values (MPH)

Trait[a]	**Set**[b]	**LG**	**Pos**[c]	**LG**	**Pos**	**LOD**	**A_i**[d]	**A_j**	**AA_{ij}**	**$Vp_{(AAij)}$**[e]
Oil	DH	N2	99.5	N10	21.0	7.1			-0.359***	2.54
Oil	DH	N3	75.8	N6	41.0	4.2			0.268***	1.42
Oil	DH	N6	55.4	N8	20.4	4.0			-0.249***	1.22
Oil	DH	N7	30.2	N8	42.4	6.8			-0.238**	1.12
Oil	DH	N11	0.0	N19	37.5	5.9		0.29***	-0.183**	0.66
Oil	DH	N11	9.3	N17	35.4	4.4			0.258***	1.31
Oil	DH	N14	0.0	N18	0.0	4.0			-0.267***	1.41
Oil	DH	N18	2.0	N19	53.1	2.2			0.199**	0.78
Oil	TC	N2	14.0	N17	83.3	2.9			-0.102***	2.04
Oil	TC	N2	152.5	N6	18.6	2.6			-0.095**	1.77
Oil	TC	N2	152.5	N9	46.4	4.0			0.119***	2.78
Oil	TC	N4	74.1	N9	40.4	2.8			0.113**	2.51
Oil	TC	N9	0.0	N17	66.1	2.9			0.101**	2.00
Oil	TC	N9	17.0	N12	29.6	7.0		0.20**	0.093**	1.70
Oil	TC	N9	96.6	N14	44.0	3.6			0.118***	2.73
Oil	TC	N11	69.6	N17	4.0	5.9			0.156***	4.78
Oil	TC	N13	56.8	N19	79.7	7.6	0.20***		0.103***	2.08
Oil	TC	N13	64.8	N15	116.9	7.9	0.24***		0.107***	2.25
Oil	TC	N15	46.6	N18	25.8	3.9			0.109***	2.33
Oil	TC	N15	42.6	N21	4.0	3.2			-0.110**	2.38
Oil	MPH	N1	70.4	N2	53.0	3.5			-0.149***	4.92
Oil	MPH	N2	123.3	N5	14.2	6.8		0.36***	-0.155***	5.33
Oil	MPH	N3	0.0	N9	13.0	3.8			-0.133***	3.92

For abbreviations see page 97

Table 33/Continued from page 93

Trait[a]	Set[b]	LG	Pos[c]	LG	Pos	LOD	A_i[d]	A_j	AA_{ij}	$Vp_{(AAij)}$[e]
Pro	DH	N1	35.7	N16	63.9	4.1			-0.196***	3.82
Pro	DH	N2	168.5	N12	12.2	3.3			-0.211***	4.43
Pro	DH	N4	24.9	N17	112.1	5.8			0.217***	4.69
Pro	DH	N6	49.2	N10	68.9	4.0		-0.17***	-0.130**	1.68
Pro	DH	N7	30.2	N8	51.0	3.6			0.187***	3.48
Pro	DH	N12	23.0	N19	12.2	3.5			-0.160**	2.55
Pro	DH	N13	29.0	N13	121.5	3.4			-0.163**	2.64
Pro	TC	N7	7.0	N19	107.8	3.7			0.096***	4.39
Pro	TC	N7	24.2	N9	107.4	6.5	-0.22***		0.099**	4.67
Pro	TC	N17	53.7	N19	60.1	3.5			-0.118***	6.64
Pro	MPH	N2	51.4	N21	0.0	2.2			-0.078**	2.55
Pro	MPH	N2	113.5	N5	16.2	5.6		-0.20***	0.112***	5.26
Pro	MPH	N6	45.0	N10	0.0	3.0			0.091***	3.47
Pro	MPH	N9	0.0	N11	38.3	3.2			-0.083**	2.89
Pro	MPH	N9	47.1	N13	19.0	5.1			0.105***	4.62
Pro	MPH	N14	62.7	N14	134.4	5.1			-0.139***	8.10
Pro	MPH	N14	102.1	N19	101.1	3.5			0.084***	2.96
Pro	MPH	N15	47.6	N18	7.2	2.7			-0.072**	2.17
Pro	MPH	N15	123.9	N19	87.1	3.4			0.080***	2.68
Pro	MPH	N18	15.2	N18	47.9	2.7			0.111**	5.17
GLS	DH	N1	37.7	N20	30.0	3.7			-1.757***	0.50
GLS	DH	N2	123.3	N5	74.1	3.7			-1.633***	0.43
GLS	DH	N3	102.1	N11	43.2	2.9			1.405***	0.32
GLS	DH	N4	24.9	N15	59.4	5.3			1.953***	0.62
GLS	DH	N5	76.3	N13	141.4	3.5			-1.607***	0.42
GLS	DH	N6	94.8	N10	15.0	2.9			-1.419**	0.33
GLS	DH	N9	12.1	N17	49.7	2.8			-1.437***	0.33
GLS	DH	N15	32.6	N19	40.0	4.6			1.351***	0.30

For abbreviations see page 97

Table 33/Continued from page 94

Trait[a]	Set[b]	LG	Pos[c]	LG	Pos	LOD	A_i[d]	A_j	AA_{ij}	$Vp_{(AAij)}$[e]
GLS	TC	N1	62.4	N17	22.1	4.3			0.873***	0.92
GLS	TC	N2	156.5	N20	6.0	3.7			-0.863***	0.90
GLS	TC	N3	55.5	N16	63.9	3.2			-0.635**	0.49
GLS	TC	N4	19.1	N15	59.4	4.8			0.813***	0.80
GLS	TC	N6	10.6	N8	10.0	3.6			-0.935***	1.05
GLS	TC	N6	53.2	N9	26.7	4.9			0.696**	0.58
GLS	TC	N8	32.2	N13	111.5	4.3			0.957***	1.10
GLS	TC	N10	17.0	N10	64.9	3.8			-0.832***	0.83
GLS	MPH	N1	114.2	N14	102.1	4.8			-0.770***	3.43
GLS	MPH	N3	0.0	N13	0.0	3.5			-0.603**	2.10
GLS	MPH	N4	4.0	N9	38.7	3.3			-0.706**	2.89
GLS	MPH	N5	97.9	N18	87.6	3.3			-0.641***	2.38
GLS	MPH	N7	7.0	N16	65.9	6.7		1.84***	0.954***	5.27
GLS	MPH	N11	14.1	N19	101.8	12.0	2.58***		0.858***	4.26
GLS	MPH	N15	47.6	N16	96.4	4.5			0.735***	3.13
GLS	MPH	N18	91.6	N20	30.0	2.9			0.685***	2.72
C22:1	DH	N1	6.0	N11	11.5	2.9			1.145***	1.72
C22:1	DH	N2	72.3	N7	30.2	2.8			1.063***	1.48
C22:1	DH	N2	156.5	N15	113.9	4.3			1.368***	2.45
C22:1	DH	N3	33.5	N8	34.4	64.6		-6.92***	-0.871**	0.99
C22:1	DH	N11	9.5	N17	35.4	3.9			1.140***	1.70
C22:1	DH	N13	0.0	N19	107.8	7.2		1.65***	1.045**	1.43
C22:1	DH	N16	31.9	N20	30.0	5.3			1.165***	1.78
C22:1	TC	N2	4.0	N6	0.0	3.0			-0.493**	1.66
C22:1	TC	N4	88.1	N11	14.1	8.9			-0.816***	4.55
C22:1	TC	N5	38.2	N11	22.3	5.1			-0.578***	2.28
C22:1	TC	N6	49.0	N14	44.0	4.0			0.463***	1.47
C22:1	TC	N6	94.8	N10	74.8	4.3			0.611***	2.55
C22:1	TC	N7	6.0	N13	129.4	3.7			0.472***	1.52
C22:1	TC	N8	20.0	N13	129.4	3.8			0.466***	1.48
C22:1	TC	N8	34.4	N16	117.9	53.0	-5.40***		0.725***	3.59

For abbreviations see page 97

Table 33/Continued from page 95

Trait[a]	Set[b]	LG	Pos[c]	LG	Pos	LOD	A_i[d]	A_j	AA_{ij}	$Vp_{(AAij)}$[e]
C22:1	TC	N8	34.4	N12	31.6	58.5	-5.56***		-0.480**	1.57
C22:1	TC	N8	40.4	N19	87.1	6.4			-0.473**	1.53
C22:1	TC	N9	49.1	N14	106.1	3.7			-0.558***	2.13
C22:1	TC	N13	129.4	N19	52.0	3.9			0.481***	1.58
C22:1	TC	N15	46.6	N16	49.9	3.3			0.488**	1.63
C22:1	MPH	N1	74.8	N9	0.0	3.4			0.411***	2.55
C22:1	MPH	N1	95.9	N17	16.1	6.7			0.548***	4.53
C22:1	MPH	N2	166.5	N13	74.0	2.2			-0.357**	1.92
C22:1	MPH	N4	84.1	N11	61.6	3.2			-0.435***	2.85
C22:1	MPH	N5	14.2	N18	0.0	3.9			0.479***	3.46
C22:1	MPH	N5	74.3	N14	116.1	4.0			-0.457***	3.15
C22:1	MPH	N5	78.3	N13	72.0	3.0			0.320**	1.54
C22:1	MPH	N6	2.0	N17	105.3	4.7			0.463***	3.23
C22:1	MPH	N6	20.7	N18	66.0	6.8			-0.615***	5.70
C22:1	MPH	N6	53.2	N18	91.6	2.7			0.367**	2.03
C22:1	MPH	N13	49.0	N21	8.0	2.4			-0.352**	1.87
C22:1	MPH	N13	133.4	N18	91.6	3.7			0.423***	2.70
C22:1	MPH	N15	46.6	N20	20.0	4.1			0.501***	3.79
Sin	DH	N2	73.5	N9	19.0	12.9		-0.18***	-0.072**	0.76
Sin	DH	N2	73.5	N16	77.9	4.1			0.101***	1.49
Sin	DH	N3	2.0	N17	124.1	2.5			-0.077**	0.86
Sin	DH	N4	86.1	N18	13.2	2.8			0.085**	1.05
Sin	DH	N5	2.2	N13	74.0	4.8			-0.079**	0.91
Sin	DH	N9	8.0	N14	58.7	3.8			0.092***	1.23
Sin	DH	N13	33.3	N17	6.0	3.2			-0.086**	1.08
Sin	DH	N15	21.7	N15	120.9	4.4			0.114***	1.89
Sin	DH	N16	18.4	N18	61.4	8.4	-0.08**	0.14***	-0.081**	0.96

For abbreviations see page 97

Table 33/Continued from page 96

Trait[a]	Set[b]	LG	Pos[c]	LG	Pos	LOD	A_i[d]	A_j	AA_{ij}	$Vp_{(AAij)}$[e]
Sin	TC	N4	23.0	N16	82.0	4.5			-0.075***	6.28
Sin	TC	N7	17.0	N8	32.2	9.6		0.16***	0.060**	4.02
Sin	TC	N7	30.2	N17	103.3	3.6			-0.071***	5.63
Sin	TC	N13	0.0	N17	74.1	3.5			-0.056**	3.50
Sin	TC	N15	127.9	N18	25.8	4.2			0.062***	4.29
Sin	MPH	N2	49.4	N6	81.4	2.8			-0.055**	2.92
Sin	MPH	N2	162.5	N6	92.8	5.1			0.089***	7.65
Sin	MPH	N3	65.3	N7	30.2	4.3			0.070***	4.74
Sin	MPH	N3	91.8	N19	87.1	2.6			0.062**	3.71
Sin	MPH	N4	0.0	N10	80.8	3.6			0.069***	4.60
Sin	MPH	N5	42.2	N17	110.1	2.8			-0.060***	3.48
Sin	MPH	N9	105.4	N21	8.0	4.5			-0.072***	5.01
Sin	MPH	N13	119.5	N17	70.1	7.1			-0.083***	6.66
Sin	MPH	N18	87.6	N19	55.1	2.5			0.054**	2.82

[a] Oil, Pro, GLS, C22:1, Sin: oil [%], protein [%], glucosinolate [µmol/g], erucic acid [%], and sinapine [mg/g], respectively
[b]DH, TC, MPH: doubled haploid population, testcross hybrid population, and midparent heterosis dataset, respectively
[c]Positions are measured from the beginning of the linkage group in cM
[d]A_i, A_j: main effects at loci i and j; AA_{ij}: epistatic interaction effect between loci i and j
* Significant at $P = 0.05$; ** Significant at $P = 0.005$; *** significant at $P = 0.001$;
[e]Vp: Explained phenotypic variance [%]

3.8 Clustering of QTL in the Rapeseed Genome

In total 14 traits analysed in the field and the green house trials were studied in the doubled haploid and the testcross populations and with the midparent heterosis data. The QTL mapping in the doubled haploid population resulted in the detection of 92 QTL, while in the testcross population and the midparent heterosis data were mapped 35 and 38 QTL, respectively. The detected QTL were not randomly distributed across the genome (Table 34, Fig 10, 12, 14). On some of the linkage groups, like N2 and N4 no QTL were localised. Just a single QTL was mapped on linkage group N14, while an apparent clustering of QTL affecting morphological, phenological and seed quality traits was observed at the upper part of N11, lower part of N12 and the middle region of N19 (Fig. 8, 10, 12). A cluster of QTL with an influence on oil-, erucic acid-, and sinapine content was mapped on linkage group N8 (Fig. 12). The three QTL for seeds per silique (Table 18), mapped in the doubled haploid population, coincided with QTL for thousand kernel weight (Fig. 8). In all cases the additive

effects were with opposite signs, partly explaining the negative correlation observed between these two yield components. Four out of five QTL for duration of flowering, mapped in the doubled haploid poplation, were congruent with QTL for end of flowering. In all cases the additive effects were with the same sign, which was in agreement with the positive correlation between duration and end of flowering. The largest QTL for plant height identified in the doubled haploid population clustered together with QTL influencing beginning and end of flowering (Fig 12).

Table 34 Number of QTL per linkage group, identified in the doubled haploid (DH), and the testcross (TC) populations, and with the midparent heterosis data (MPH) for all studied traits

		QTL number			
LG[a]	**Size [cM]**	**DH**	**MPH**	**TC**	**Total**
N1	100.5	6	0	3	9
N2	141.6	0	0	0	0
N3	98.7	4	2	2	8
N4	77.9	0	0	0	0
N5	138.7	9	3	4	16
N6	85.2	0	1	0	1
N7	27.9	4	0	4	8
N8	50.8	4	2	3	9
N9	92.8	6	1	3	10
N10	103.0	6	1	1	8
N11	64.1	9	5	4	18
N12	33.3	6	3	3	12
N13	121.7	7	2	1	10
N14	117.4	0	2	0	2
N15	114.3	4	0	0	4
N16	104.9	7	2	3	12
N17	111.5	3	1	1	5
N18	82.4	2	0	0	2
N19	98.5	13	7	4	24
LG5	24.7	0	0	0	0
LG10	8.5	0	0	0	0

[a]LG: name of the linkage group

4. Discussion

4.1 The Linkage Map

On the basis of linkage analysis in 96 doubled haploid lines, in case of the primary map, 363 marker loci were assembled in 24 linkge groups and three additional markers remained unlinked. Based on an alignment with previously established maps (Lowe et al. 2004; Piquemal et al. 2005; Sharpe and Lydiate, unpublished data, Uzunova et al. 1995, extended version of the map) four linkage groups N2a, N2b, N14a, and N14b could be unambiguously grouped in two pairs, representing linkage groups N2 and N14. Even after the grouping the number of linkage groups (22) was greater than the number of chromosomes in *B. napus* (n = 19) with three groups that could not be aligned to any of the N groups. This observation is most probably a consequence of incomplete coverage of the entire genome. Reducing the LOD threshold from 4.0 to 3.0 and even to 2.5 did not lead to the connection of the three small groups, to any of the N linkage groups, representing the 19 rapeseed chromosomes. This may indicate that the unassigned linkage groups are rather due to lack of markers at these regions than a result of disconnecting real linkage associations by an application of a high LOD threshold.

In a study on a consensus linkage map construction, Lombard and Delourme (2001) estimated a probable range of the rapeseed genome length from 2,127 cM to 2,480 cM. Considering the mean estimated from these values our map, with a length of 1,916.1 cM, covers 83.2% of the rapeseed genome. More markers should be used to obtain better genome coverage. Applying a consensus mapping approach as well, Piquemal et al. (2005) developed a linkage map of *B. napus*, which covered 2,619 cM, which was beyond the range estimated by Lombard and Delourme. Apart from the consensus maps of Lombard and Delourme (2001), and Piquemal et al. (2005) the longest published linkage map of rapeseed was developed by Cheung et al. (1997), covering 1,954.7 cM for 19 major linkage groups and 2,124.9 cM by including ten unassigned fragments of less than four markers. On the other hand three maps published by Parkin et al. (1995) and Sharpe et al. (1995), which ranged from 1,606 cM to 1,741 cM are considered as rather complete (Lombard and Delourme 2001). A critical point here is that the reported map sizes are not easily comparable, since they are dependent on the degree of genome coverage by marker loci, on the size and the type of the mapping population, on the mapping function applied, and on the recombination frequencies, which are influenced by the genetic diversity of the parents and/or environmental effects on meiosis (Ferreira et al. 1994).

A high and significant correlation of r = 0.82 ($P = 10^{-4}$) between the length and the number of markers of the linkage groups is an indication that the markers are relatively evenly distributed across the linkage groups. Similar results are reported by Lombard and Delourme (2001) and Foisset et al. (1996). Despite the apparent linear relationship between the marker number and the linkage group size, non-uniform marker distributions were observed eighter due to clusters or gaps of markers. Uneven marker density has been observed in a number of studies (Foisset et al. 1996; Parkin and Lydiate 1997; Uzunova et al. 1995). The differences in the size of the linkage groups and the clustering of the markers may be partly due to differences in the physical chromosome length and the recombination frequencies in different parts of the genome. Röbbelen (1960) reported large differences between *B. oleracea* and *B. napus* chromosomes in a cytogenetic study. In a high density map of tomato Tanksley et al. (1992) observed highly increased marker densities near centromeres and part of the telomeres, indicating recombination suppression in these regions. The different marker number on linkage groups could be partly attributed to differences in the distribution of repetitive DNA, since predominantly microsatellite markers were used in the mapping. This provides also an explanation why some linkage groups consisted mostly of AFLP markers and shows that better genome coverage could be obtained using combinations of different marker systems.

4.2 Duplicated Regions and Dominant Markers

Brassica napus is an amphidiploid species with 19 chromosome pairs. Cytological evidence indicates that it has been formed by a spontaneous hybridization of *B. rapa* (n = 10) and *B. oleracea* (n = 9) (Prakash and Hinata 1980), or close relatives to *B. montana* from the *B. oleracea* complex (Song and Osborn 1992). In a mapping population developed from a cross between a resynthesized *B. napus* and a “natural” rapeseed, Parkin et al. (1995) observed that the majority of loci exhibit disomic inheritance of parental alleles, demonstrating that *B. rapa* chromosomes were pairing with A-genome homologues in *B. napus* and *B. olercea* chromosomes were pairing in the same way with the C-genome homologues. By this experiment the 10 A and 9 C genome linkage groups in *B. napus* were identified and it was demonstrated that the nuclear genome of rapeseed and its progenitors have remained essentially unaltered since the speciation event. The presence of the A (*B. rapa*) and the C (*B. oleracea*) genomes within amphidiploid *Brassica napus* provides a ready explanation for the presence of duplicated loci in the *Brassica napus* genome. The high level of homoeology between the A and C genomes reflected in complex RFLP patterns, identifying in most cases an even number of loci (Ferreira et al. 1994; Parkin et al. 1995;

Sharpe et al. 1995; Uzunova et al. 1995). Duplications within the diploid genomes were reported, as well (Chyi et al. 1992; Lagercrantz and Lydiate 1996; Slocum et al. 1990; Song et al. 1991). In our study the duplicated loci mapped by SSR primer pairs, amplifying more than one polymorphic locus, should be considered as the lowest limit of duplications in the rapeseed genome since most of the primer pairs showing polymorphisms amplified a polymorphic locus, which was accompanied by a monomorphic peak.

The large number (122 of 243) of dominant microsatellite markers can be partially attributed to the high level of duplications, as well. As explained by Uzunova et al. (1995) directly after the speciation event leading to the diploid ancestors of an amphidiploid species or following duplication within the diploid genomes the duplicated loci would have had identical alleles. If an allele from only one of the duplicated loci mutate then a dominant marker will appear. The second allele at this locus will be masked by the monomorphic product from the duplicated locus. A mutation creating two new alleles at one locus leads to a codominant marker. A mutated and an original allele at each of the duplicated loci cause a segregation ratio of 3:1 for the common allele, while the other two alleles could be scored as dominant markers. Such a pattern was detected several times in our analysis. Similar results could be observed in case of nonreciprocal homoeologous translocations (Sharpe et al. 1995) in the F_1 hybrid from which the doubled haploid population was developed. This specific kind of mutation leads to untypical banding patterns in a DH-population. In the lines, which carry a nonreciprocal homoeologous translocation for some loci both of the parental alleles are observed, while in their homoeologous loci no allele is detected. Markers with such banding patterns were excluded from the analyses in our study, in order to prevent the formation of pseudolinkage-groups (Sharpe et al. 1995).

4.3 Disturbed Segregations in the Rapeseed Genome

One hundred and fourteen (31.4%) of the mapped markers showed a significant deviation from the expected 1:1 segregation ratio. Such disturbed segregations are typical for microspore culture developed doubled haploid populations of a wide spectrum of crop plants as reviewed by Foisset and Delourme (1996). A common opinion is that this phenomenon is a result of specific parental alleles favourable for in vitro androgenesis or the subsequent plant regeneration. This hypothesis is supported by the observation that no or very low levels of disturbed segregation are detected in corresponding F_2 populations (Lombard and Delourme 2001; Piquemal et al. 2005; Uzunova et al. 1995). On the other hand Ferreira et al. (1994) compared a doubled haploid map to a partial map constructed with a common set of markers

for an F_2 population derived from the same F_1 plant. Deviation from Mendelian segregation ratios was observed for 30% of the markers in the doubled haploid population, while in the F_2 population 24% of the markers showed disturbed segregation, which was lower, than in the DH-population, but still higher than statistically expected. Moreover different loci showed deviation from the expected ratios in the two populations. The authors concluded that not only the response to microspore culture but also other factors - environmental and/or random effects - influence the selection of specific genotypes. This could provide an explanation why in some populations, that were developed from parents with very different responses to androgenesis, equal number of loci with non-Mendelian segregation in favour of the alleles of one or the other parent is observed (Foisset et al. 1996). An intensive selection at tissue culture level or at later developmental stages, caused by a specific response to microsposre culture or other environmental and /or random effects, in favour of only one of the parents can lead to a strong bias in QTL mapping, since the trait distribution will be not normal if a locus determining the trait is linked to a factor causing a selection process. Such an effect was not observed in our study as the distribution of all polygenic determined traits did not deviate from the normal distribution. In total 10 blocks of markers with disturbed segregation were identified on 10 different linkage groups, which implies a minimum number of 10 factors segregating in the mapping population that might have an influence on in vitro androgenesis and/or plant regeneration, and further development. The favourable alleles of 5 of these factores were inherited from ‘Express’, while the other 5 were contributed by the resynthesized parent. This shows that different loci are involved in the determination of androgenic capacity of the two parents or of other factors causing disturbed segregation.

4.4 Genetic Basis of Heterosis

The plant material used in this study and the specific crossing scheme were chosen to optimize the ability to detect QTL contributing to heterosis, to estimate their effects and assess the degree of dominance and to identify whether they are involved in digenic epistatic interactions. The QTL mapping in the doubled haploid and testcross populations allowed the identification of additive and nonaditive gene actions. Important was the choice of a tester. The tester ‘MSL-Express’ is a male sterile version of the cultivar ‘Express’ used as a parent for the original cross from which the doubled haploid population was developed, meaning that the testcross population is genetically equivalent to a BC_1 population. The use of one of the parents as a tester for hybrid production provided the opportunity for a straightforward determination of the genetic effects. If an independent tester, that may introduce alleles

unrelated to the parental alleles, is used then the genetic additive effects are confounded and it is impossible to distinguish the additive and dominance effects in the testcrosses. Mei et al. (2003) faced such a problem in studying the molecular basis of heterosis in a RIL and two TC populations of rice with independent testers and concluded that some uncertainty remains with respect to the reliability of the dissection of gene effects mainly as a result of the unknown homology and dominant/recessive relationship between alleles from the RIL and the tester. The genotypes of backcross hybrids, on the other hand, can be unambiguously deduced from the marker information of the parental doubled haploid or recombinant inbred lines, i.e. homozygous for alleles from the recurrent parent or heterozygous. Therefore the genetic effects in a backcross population can be more precisely defined in contrast to those in a testcross with an independent tester (Mei et al. 2005). The disadvantages of the use of a backcross population are that only 50% of the possible heterosis is realized and that the genetic variance is reduced. In our study the QTL main effects detected in the doubled haploid population represented additive effects (a), the midparent heterosis data gave an estimation of the dominance effects (d), while the effects identified with testcross hybrid data contained both additive and dominance effects: -(a + d) or (a – d), depending on whether the donor or the recurrent parent carried the dominant allele. Similarly the epistatic interactions identified in the doubled haploid population represented additive x additive epistasis, while these detected with midparent heterosis and testcross hybrid data were complex mixtures of all possible epistatic interactions including additive x additive, additive x dominance, dominance x additive, and dominance x dominance epistatsis (Kearsey and Pooni 1996).

In the current study an apparent difference was observed between the levels of heterosis of the seed quality traits and the remaining traits analysed. For that reason I summarised separately the results of quantitative trait loci analysis of heterotic and qualitative, nonheterotic traits.

4.4.1 Main Effect QTL Mapping

In main effect QTL mapping of heterotic traits including early fresh biomass, grain yield, thousand kernel weight, seeds per silique, siliques per unit area, plant height, beginning, end and duration of flowering (Table 35) 60 QTL were identified in the doubled haploid population, showing additive effects. Twenty seven of them were congruent with QTL identified with the other datasets, allowing the assessment of the degree of dominance. Of the 27 QTL with dominance effects, identified in the midparent heterosis data, 11 showed partial dominance, 2 displayed full dominance, and 14 exhibited overdominance. In heterosis studies

of maize Frascaroli et al. (2007) observed that QTL for traits with low heterosis were prevailingly in the additive x dominance range, while QTL for highly heterotic traits had effects in the dominance x overdominance range. Similarly in our study early fresh biomass and grain yield, which were the traits with the highest level of heterosis were among the traits with the largest number of loci showing overdominance (Table 36). Surprisingly end of flowering and thousand kernel weight, which showed low levels of heterosis, included only overdominant loci and three overdominant out of four loci with dominance effect, respectively. For seed quality traits, which showed very low or no heterosis on population level, loci with dominance effects were nevertheless identified (Table 35) but they were considerably lower in number in comparison to the rest of the studied traits and fitted prevailingly in the additive x dominance range with only 3 loci exhibiting overdominance.

Table 35 Summary of quantitative trait loci analysis of heterotic and quality traits

	Main effect QTL									Epistatic QTL		
	Nr. of QTL[b]			Nr. of coincidenig QTL[c]			Nr. of QTL with dominance effects			Nr. of epistatic interactions[d]		
Trait[a]	DH	MPH	TC	DH/ MPH	DH/ TC	MPH/ TC	Partial-	Full-	Over-	DH	MPH	TC
Heterotic traits												
9	60	27	22	17	11	5	11	2	14	60	73	56
Quality traits												
5	32	11	13	6	7	2	7	1	3	39	39	45

[a]Number of traits analysed
Heterotic traits: early fresh biomass, grain yield, thousand kernel weight, seeds per silique, siliques per square decimeter, plant height, beginning of flowering, end of flowering, and duration of flowering
Quality traist: oil, protein, glucosinolate, erucic acid, and sinapine content
[b]Number of main effect QTL detected in the doubled haploid population (DH), the midparent heterosis data (MPH) and in the testcross hybrid population (TC).
[c]Coinciding QTL: Number of QTL, identified in more than one dataset
[d]Number of digenic epistatic interactions

Our results show that all levels of dominance in the range from partial to overdominance play a role in the expression of heterosis in the rapeseed population studied. Considering all heterotic traits together no specific genetic effect was predominant as overdominance accounted for 51% of the loci showing dominance, while the remaining 49% exhibited partial to full dominance. If the difference in the phenotypic variance explained is considered then with 71.2% the fourteen QTL showing overdominance explained a much larger portion of the

phenotypic variance than the 29.6% explained by the thirteen QTL exhibiting partial to full dominance.

A doubled haploid population and its corresponding testcrosses were used for QTL analysis in the current study. The DH population was developed from microspores of a single F_1 hybrid after only one cycle of meiosis, meaning that a high level of linkage disequilibrium is retained. As a result the observed overdominance at single loci in our study can not be distinguished from the pseudo-overdominance generated from a close linkage of genes with partial or full dominance in repulsion phase (Crow 1952). Fine mapping at these loci can help to break a possible linkage and to determine whether the overdominance observed was due to pseudo-overdominance or not.

In a pioneering study of heterosis at the molecular level in maize Stuber et al. (1992) mapped QTL associated with 7 major traits and suggested that overdominance and/or pseudo-overdominance play a significant role in heterosis. The largest QTL for yield detected on chromosome 5 in that experiment was further dissected by Graham et al. (1997), who by fine mapping revealed that the seemingly overdominant action of the original QTL is actually psedo-overdominance. To reduce incidences of pseudo-overdominance Lu et al. (2003) used a maize population derived from a F_2 population by three generations of random mating to assess the degree of dominance of 4 heterotic traits. Despite using a random mated population 24 of 28 QTL for grain yield nevertheless showed overdominance. On the other hand, the majority of QTL for the other traits showed only partial dominance. Similar results were reported in a more recent study in maize by Frascaroli et al. (2007) who observed partial to full dominance for seedling emergence, days to pollen shedding, anthesis silking interval and kernel weight, whose heterosis levels ranged from 5% to 34%. For highly heterotic traits as seedling weight, plant height, grain yield, and number of kernels per plant, whose heterosis levels ranged from 52% to 239%, prevailingly overdominance was observed.

Rapeseed is a partially allogamous crop with considerably lower levels of heterosis than maize. The highest levels of heterosis which were detected for early fresh biomass and grain yield were 33% and 30%, respectively, compared to heterosis of over 100% frequently observed in maize. Nevertheless in rapeseed 3 out of 4 dominant loci for early fresh biomass and 2 out of 4 dominant loci for grain yield showed overdominance. High levels of overdominance for reproductive and morphological traits were reported in rice (Li et al. 2001; Luo et al. 2001; Mei et al. 2003; Mei et al. 2005) and tomato, as well (Semel et al. 2006). These results show that although it is highly possible that there exist different genetic mechanisms, which explain heterosis for specific traits in different organisms, the proposal of

Semel et al. (2006) that the association of overdominant QTL for traits determining higher reproductive fitness was selected for in evolution and was domesticated by man to improve yields of crop plants, could be true.

The number of QTL detected with the testcross and the midparent heterosis data was considerably smaller and explained lower percentages of the phenotypic variation than the number of QTL detected in the doubled haploid population. The main reason for the reduced power of detection could be attributed to the fact that in a doubled haploid population the difference between the QTL genotypes tested for significance represents two times the additive effect, while with the midparent heterosis data this difference, representing the dominance effect, is only one time the additive effect in case of full dominance or smaller in case of partial dominance. If an overdominance is observed the difference will exceed the additive effect but a dominance ratio of 2 is necessary to reach the effect tested in a doubled haploid population. In our study the highest dominance ratios observed were 2.9 and 3.3, both of them detected for early fresh biomass. For most of the other traits the dominance ratio in case of overdominance was lower than 2. These results indicate that a certain bias in the number of QTL with dominance effects exists in direction of QTL showing overdominance, since the small effects of QTL exhibiting partial dominance are these, which remain under the power of detection. An impediment in detecting QTL in the testcross hybrid population is the so called “masking effect of the tester” (Gallais and Rives 1993). Most probably a number of loci with positive additive effects, where no corresponding QTL were detected with the midparent heterosis or the testcross hybrid data, exhibit full or partial dominance with a magnitude lower than the power of detection in the QTL mapping. The failure to detect such QTL with testcross hybrid data results from the fact that in case of a dominant allele, carried by the recurrent parent, the effect represents a difference between the additive and dominance effects at this locus and the two effects cancel each other in case of full dominance or the resulting effect is too low to be detected in case of partial dominance. The opposite situation explains why in some cases QTL were observed in the testcross population data and not in the other two datasets. In case of an increasing dominant allele coming from the donor parent the genetic effect for this locus in the testcross population represents the sum of the additive and dominance effects, which are too low to be detected separately in the other datasets.

The reduction in the power of detection due to testing smaller differences with midparent heterosis and testcross hybrid data is compensated to some extend by the lower total genotypic variance in these datasets compared to the variance in the doubled haploid

population, as the power of detection for a QTL depends on the ratio between the variance explained by the QTL to the total variance of the trait (Lander and Botstein 1989).

4.4.2 Epistatic QTL Mapping

A large number of epistatic interactions were detected with the three different datasets for both heterotic and seed quality traits (Table 35), meaning that epistatic interactions play an important role not only in explaining phenotypic variation in the performance of the doubled haploid lines but also that epistasis contributes to the expression of heterosis in rapeseed, explaining as large and even larger portions of the phenotypic variation than the main effect QTL (Table 36). The epistatic interactions identified with the midparent heterosis data are these, which exclusively contribute to the expression of heterosis. For early fresh biomass and grain yield the phenotypic variance explained by the main effects in the midparent heterosis data was 14.8% and 18.1%, while with 39.3% and 36.6% (Table 36) the phenotypic variance explained by the digenic epistatic interactions was about twice as large. The difference between the phenotypic variance explained by the main effect QTL and epistatic interactions in the midparent heterosis data was even more pronounced for seeds per silique and plant height where 4.3% and 7.8%, respectively, were explained by the main effects, while the epistatic interactions accounted for 51.8% and 39.9% of the phenotypic variance, respectively.

In general the magnitude of the epistatic effects was lower than that of the main effects, but the epistatic QTL mapping identified much larger number of loci contributing to trait expression, than the single-QTL model. For example the main effect QTL mapping with midparent heterosis data for the traits showing the highest levels of heterosis early fresh biomass and grain yield resulted in the detection of 3 and 4 QTL, respectively, while 11 digenic epistatic interactions were identified for early fresh biomass and 9 for grain yield (Table 36). According to Li et al. (2001) the epistatic interactions could be classified in three groups depending on the main effects of the loci involved. Epistatic interaction between two loci with significant main effects represents type I, between a significant main effect QTL and a locus without main effect is type II, and between two background loci with no significant main effects is described as type III. Our results confirmed those of Li et al. (2001), Luo et al. (2001) and Yu et al. (1997) in rice, showing that epistasis does not necessarily occur between main effect QTL. We observed just a single epistatic interaction of type I, 43 out of 312 (13.8%) were of type II and the remaining 268 (85.9%) were of type III. A larger part 61% of the type II interactions were detected in the doubled haploid population, which could be explained with the larger number of main effect QTL identified in this dataset. The remaining

39% were approximately equally distributed between midparent heterosis and testcross hybrid data with 17% and 22%, respectively.

Table 36 Summarized information for the number and congruency of main effect and epistatic QTL identified with the doubled haploid (DH), and testcross (TC) populations, and the midparent heterosis data (MPH)

				Dominance[e]			Epistasis[f]						
Trait[a]	Set[b]	mQTL[c]	Overlap[d]	p	f	o	I	II	III	epQTL	Vp(m)[g]	Vp(e)	Vp(t)
EFB	DH	5	2					2	10	12	31.80	34.10	65.90
EFB	MPH	3	2	1		3			11	11	14.80	39.30	54.10
EFB	TC	3	2					2	8	10	14.60	38.10	52.70
GY	DH	6	3					4	2	6	32.70	8.47	41.17
GY	MPH	4	3	2		2			9	9	18.10	36.60	54.70
GY	TC	1	0					1	6	7	2.30	33.40	35.70
TKW	DH	8	4					3	5	8	27.80	20.90	48.70
TKW	MPH	3	3	1		3			2	2	26.50	11.90	38.40
TKW	TC	4	4					1	5	6	28.70	28.40	57.10
S/Sil	DH	3	3					1	7	8	25.50	19.30	44.80
S/Sil	MPH	2	2	2					14	14	4.30	51.80	56.10
S/Sil	TC	2	1						2	2	18.30	12.00	30.30
Sil/dm^2	DH	7	0					1	8	9	32.70	33.50	66.20
Sil/dm^2	MPH	0	0						2	2	0.00	10.50	10.50
Sil/dm^2	TC	1	0						1	1	6.30	8.00	14.30
PH	DH	7	1					1		1	27.70	3.30	31.00
PH	MPH	2	1		1	1		1	4	5	7.80	39.30	47.10
PH	TC	2	0					1	2	3	16.50	17.90	34.40
BF	DH	9	7					1	3	4	50.40	12.50	62.90
BF	MPH	4	4	4	1	1		1	7	8	15.00	27.70	42.70
BF	TC	6	5					2	2	4	22.70	20.20	42.90
EF	DH	9	2					1	5	6	34.50	19.20	53.70
EF	MPH	3	2			3			11	11	17.60	40.30	57.90
EF	TC	3	0						9	9	21.00	49.40	70.40
DF	DH	6	2					3	4	7	22.00	16.40	38.40
DF	MPH	2	2	1		1		1	10	11	10.50	44.70	55.20
DF	TC	0	0						14	14	0.00	54.70	54.70
Oil	DH	11	5					1	7	8	29.60	10.50	40.10
Oil	MPH	3	2	1	1			1	2	3	16.00	14.20	30.20
Oil	TC	3	2					3	9	12	37.80	29.40	67.20
Pro	DH	9	1					1	6	7	31.20	23.30	54.50
Pro	MPH	1	0	1				1	9	10	4.30	39.90	44.20
Pro	TC	3	1					1	2	3	16.10	15.70	31.80

For abbreviations see page 109

Table 36/Continued from page 108

Trait[a]	Set[b]	mQTL[c]	Overlap[d]	Dominance[e]			Epistasis[f]			epQTL	Vp(m)[g]	Vp(e)	Vp(t)
				p	f	o	I	II	III				
GLS	DH	5	2						8	8	26.00	3.30	29.30
GLS	MPH	3	2	1		2		2	6	8	31.00	26.20	57.20
GLS	TC	3	1						8	8	24.30	6.70	31.00
C22:1	DH	2	1					2	5	7	66.30	11.60	77.90
C22:1	MPH	2	1	1		1			13	13	26.60	39.32	65.92
C22:1	TC	1	1					2	11	13	52.90	27.48	80.38
Sin	DH	10	3				1	1	7	9	33.50	10.20	43.70
Sin	MPH	1	1	3				1	4	5	2.70	41.60	44.30
Sin	TC	3	2						9	9	14.20	23.70	37.90

[a]EFB: early fresh biomass, GY: grain yield, TKW: thousand kernel weight, S/Sil: seeds per silique, Sil/dm^2: siliques per square decimeter, PH: plant height, BF: beginning of flowering, EF: end of flowering, DF: duration of flowering, Oil: oil content, Pro: protein content, GLS: glucosinolate content, C22:1: erucic acid content, and Sin: sinapine content

[b]DH, MPH, and TC – doubled haploid line, midparent heterosis, and testcross hybrid data, respectively

[c]mQTL and epQTL – number of main effect and epistatic QTL, respectively

[d] Overlap – Number of coinciding QTL detected in more than one dataset

[e]Dominance – number of dominance effects displaying partial- (p), full- (f), and overdominance (o). The discrepancy between the sum of QTL with different dominance effects and the QTL detected in MPH is due to dominance effects calculated indirectly from the other datasets at loci not significant in MPH

[f]Epistasis I, II, III – number of first, second, and third type epistatic interactions, respectively

[g]Phenotypic variance explained by the main effect [Vp(m)], epistatic effects [Vp(e)], and the sum of them [Vp(t)]

Variation in the number of main effect QTL involved in epistasis was observed not only between the different datasets used for QTL mapping but between different traits as well. The largest number of type II epistatic interactions was identified in the doubled haploid population for grain yield, thousand kernel weight and duration of flowering, 4 out of 6, 3 out of 8, and 3 out of 7 interactions, respectively. In the current study oil content was not among the traits with the highest number of loci with significant main effect involved in epistasis. These results were in discrepancy with the results reported by Zhao et al. (2005), who identified 11 digenic interactions for oil content in a doubled haploid population developed from a cross between an European and a Chinese cultivar. Seven of these epistatic interactions were of type I, and 4 of type II. No type III interactions were detected. Considering the large number of type III interactions observed in the rapeseed population under study, our results more closely resembled the observations of Li et al. (2001) and Luo et al. (2001) in rice, who detected prevailingly type III epistatic interactions as well. Some of the loci involved in epistasis interacted with more than one locus, for example a locus for thousand kernel weight located in the doubled haploid population on N6, or loci for seeds per silique mapped on N2 and N4 with the midparent heterosis data etc. The participation of loci

in multiple digenic epistatic interactions could be a reflection of the existence of higher order epistatic interactions, meaning that the number of epistatic interactions may still be underestimated in the present study, as we restricted our analysis to digenic interactions. Currently the contribution of higher order interactions can not be estimated since there is no available software handling such a complex issue. Moreover a population of 250 doubled haploid lines is not big enough to resolve higher order epistatic interactions as pointed out by Mei et al. (2005) and Zhao et al. (2005).

4.4.3 Evidences for Epistasis on Population Level

Evidences for the influence of epistasis on the expression of heterosis were identified at population level as well. If we consider regular meiosis and no gametic selection each of the doubled haploid lines should include 50% of the two parental genomes meaning that the testcross hybrids had about twice less heterozygous loci than the parental F_1 hybrid. This provides an explanation of the twice lower average testcross midparent heterosis than the F_1 midparent heterosis observed in a number of traits e.g. early fresh biomass, grain yield, beginning of flowering, end of flowering, and duration of flowering. The heterotic values of some traits, like plant height, seeds per silique, and siliques per square decimetre, apparently deviated from the expected reduction. In case of plant height the F_1 midparent heterosis was only reduced from 16% to 14% in the average testcross midparent heterosis, while for seeds per silique even an increase was observed from 11.2% to 12.7%. In contrast the heterosis of siliques per square decimetre was reduced not twice but 10 times. For all traits deviating from the expected 50% reduction of heterosis a significant difference (P = 0.05) was observed between the MPV of ‘Express’ and ‘R53’ and the mean of the doubled haploid lines, which is a hint for the presence of epistatic interactions. For plant height and siliques per square decimetre the lower MPV in comparison to doubled haploid population mean could be a result of negative epistatic gene complexes occurring in the parental genotypes, which are broken due to recombinations in the doubled haploid lines. In contrast the reduced doubled haploid line mean of seeds per silique compared to the MPV of ‘Express’ and ‘R53’ could be due to a loss of positive epistatic interactions occurring in the two parents. In a study of rice Mei et al. (2005) concluded that the observed higher heterosis in the backcross hybrids compared to the parental F_1 is due to the elimination of heterozygous loci with negative dominance effect and additive x dominant epistasis.

4.4.4 Comparison of Epistasis in Rapeseed with Maize and Rice

The effect of epistasis on heterosis was extensively studied in maize and rice leading to contradictory results. In the studies of Lu et al. (2003), Mihaljevic et al. (2005), and Stuber et al. (1992), no significant epistasis was detected in maize by testing all possible pair-wise combinations of markers linked to the mapped QTL. Using the same approach no epistasis was detected by Xiao et al. (1995) in rice as well. On the other hand Yu et al. (1997) applying two-way analyses of variance using all possible pair-wise combinations of marker genotypes to test epistasis and Luo et al. (2001) and Li et al. (2001) using mixed linear model with background variation control to map simultaneously main and epistatic effects, reported that epistasis is a common feature of most loci associated with inbreeding depression and heterosis in rice. The latter authors pointed out the absence of an appropriate mapping methodology in the previous studies as a possible explanation for the observed discrepancy with the results in maize and those reported for rice by Xiao et al. (1995). In a study of maize Frascaroli et al. (2007) applied the same QTL model for epistasis analyses as that used by Luo et al. (2001) and Li et al. (2001). They detected only a very low number of epistatic interactions, indicating that the difference in the importance of epistasis to heterosis between the allogamous maize and autogamous rice cannot be attributed only to the different statistical approaches used. The results of our study in rapeseed, together with the available data in rice and the recent evidences for epistasis in Arabidopsis (Kusterer et al. 2007) support the hypothesis of Li et al. (2001) that epistasis for complex traits appears to be more pronounced in self-polinated than in cross-polinated crop species as in the former coadapted gene complexes with favourable epistasis between loosely linked or even unlinked loci can be more easily maintained.

4.4.5 Relationship between Heterozygosity and Hybrid Performance

The degree of the correlation between genome heterozygosity and phenotypic traits reflects the importance of overall genome heterozygosity to trait expression. In the population under study no significant correlation was observed between the overall genome heterozygosity and the testcross *per se* performance and the midparent heterosis for any of the traits analysed. There exists an evident difference between maize and rice considering such correlations according to the literature, with significant correlation coefficients between whole genome heterozygosity and trait expression observed in maize hybrids and only insignificant correlations detected in rice. In maize Frascaroli et al. (2007) and Stuber et al. (1992) reported high correlations between heterozygosity levels and phenotypic performance of testcross hybrids for the traits showing high levels of heterosis and high degrees of dominance, like

grain yield, while for the less complex traits showing lower levels of heterosis the observed correlations were reduced. Stuber attributed these results to the large number of genes responsible for the trait expression of grain yield and emphasized that traits controlled by only one or few loci are expected to show very low correlation coefficients between phenotypic performance and the level of genome-wide heterozygosity. In contrast to the correlation between heterozygosity and hybrid performance observed by Stuber et al (1992), Melchinger (1999) showed thtat high correlations between genetic distance between parents and heterosis of the respective hybrids is observed in crosses among more or less related lines. There is a weak correlation in crosses among nonrelated lines within the same gene pool and no correlation if for all crosses the parents belong to different genepools. In studies on rice Xiao et al. (1995) and Yu et al. (1997) ascribed the lack of correlation between marker heterozygosity and trait expression to the detected positive and negative dominance for most of the traits, leading to a cancelling of the positive and negative dominance effects of the QTL controlling the trait. Similar to Frascaroli et al. (2007) our QTL mapping results revealed mostly dominance effects having the same sign for a particular trait, nevertheless no significant correlation between the overall genome heterozygosity and the trait expression was observed. It was inferred that high levels of heterosis are a result of heterozygosity at certain loci and not from genome-wide hetrozygosity. In principle the correlation of a trait to overall heterozygosity suggests that no specific genes or alleles are responsible for hybrid vigour. Our QTL analysis demonstrated that there are specific loci, often showing overdominance, which are responsible for the expression of heterosis, meaning that the heterozygous status at these particular loci is much more important for the increased hybrid vigour, than the overall genome heterozygosity. Looking to the same problem from another angle we could argue that the contribution of locus-specific heterozygosity or homozygosity to heterosis could be the genetic basis of specific combining ability (Mei et al. 2005).

4.4.6 Hot Spots for QTL in the Rapeseed Genome

In the present study three genomic regions were identified, each not larger than 20 cM, which harboured overlapping QTL for a large number of traits. The upper part of linkage group N11 in the interval from 0 to 12.1 cM included 15 QTL for thousand kernel weight, seeds per silique, early fresh biomass, beginning of flowering, duration of flowering, plant height and glucosinolate (Fig. 8, 10, and 12), identified in the doubled haploid and testcross populations and midparent heterosis data. The QTL with the highest effects for seeds per silique, early fresh biomass and beginning of flowering were among the QTL located in this

region. In a genomic segment with a length of 9.2 cM on linkage group N12 12 QTL were identified in the 3 different datasets for the traits thousand kernel weight, grain yield, early fresh biomass, siliques per square decimetre, plant height, end of flowering, duration of flowering, and oil content. Among these QTL the QTL with the highest effects for grain yield, siliques per square decimetre, and the QTL for plant height with the highest dominace effect were detected. Linkage group N19 carried 7 QTL for thousand kernel weight, seeds per silique, siliques per square decimetre, and beginning of flowering identified with one of the three datasets in the interval from 52.0 cM to 74.9 cM. Of the total number of 34 QTL identified in the three genomic regions 9 QTL (26.5%) were detected in the midparent heterosis dataset, meaning that they are involved in the expression of heterosis. Most of the overlaps between the identified QTL in these 3 particular regions were due to linkage between different genes involved in the expression of different traits, but we can not exclude the possibility of pleiotropic effects of some genes on different traits simultaneously. It could be hypothesized that such genes regulate fundamental metabolic processess and affect the overall plant vigour, thus indirectly influencing a wide spectrum of agronomic traits. Similar important overlaps between QTL associated with plant vigour like QTL for seedling weight, plant height, number of kernels per plant and total grain yield were reported by Frascaroli et al. (2007), while Stuber et al. (1992) identified congruent QTL for ear leaf area, plant height, and grain yield. A possibility for spurious clustering of QTL could be the influence of a specific trait on a large number of other traits. For example a gene for extreme susceptibility to a pathogene would influence nearly all other traits. To prevent such false QTL clustring, in the current study, the genotypes, which had been strongly infected by pathogenes were considered as missing data.

QTL, which significantly influence trait expression of several important agronomic traits by their additive effects in homozygous genotypes and which strongly contribute to heterosis by their dominance effects are of great interest for further analyses. A possible strategy here would be fine mapping of selected QTL in an advanced backcross population using marker assisted selection. Subsequently, the synteny between rapeseed and *Arabidopsis* could be used for an identification of possible candidate genes for the fine mapped QTL.

4.4.7 Possible Application in Practical Breeding

Heterosis is extansively used in practical breeding for a wide spectrum of crop plants through the production of hybrid varieties. The first hybrid rapeseed variety in Germany was registered in 1995 followed by a fast development of hybrid breeding. Nowadays, hybrid

varieties represent 30% and 60% of the total rapeseed production in Europe and Germany, respectively. Nevertheless heterosis is still a 'black box' with very few knowledge about its molecular basis and causal factors.

It was observed that the ten best yielding testcross hybrids of the current study outperformed the line variety 'Express' and two of them equalled the grain yield of the comercial hybrid 'Elektra', demonstrating that the breeding for hybrid varieties has a greater potential than line breeding and that the use of resynthesized rapeseed as a parent for the production of highly heterotic crosses could be of interest.

The quantitative genetic analysis results indicated that the genetic basis of heterosis in rapeseed is very complex, which is reflected by the large number of loci involved, their wide genomic distribution, and complex epistatic relationships. The exploitation of heterosis for crop improvement using marker assisted transfer of desirable QTL alleles identified in our study is expected to be impeded by the relatively few main QTL with large dominance effects and the high number of epistatic interactions involved in the determination of heterosis. Nevertheless the 3 main-effect QTL for yield showing dominance effects, and especially the QTL for grain yield on linkage group N12, which is the QTL with the largest additive and dominance effect identified in our study, could be of interest for plant breeding.

A possible strategy for using the current results for an improvement of the utilisation of heterosis in hybrid breeding is to introgress the detected QTL alleles in lines of the complementary heterotic pools. If we consider for example grain yield, then all QTL alleles with additive effects increasing the trait could be pyramided in the lines of the genepool of 'Express' in order to increase their *per se* performance in the homozygous state, while the QTL alleles coming from the exotic resynthesized parent, which showed overdominance in the heterozygous hybrid could be introduced in the opposite heterotic pool, thus the identified QTL alleles contributing strongly to heterosis could be purposefully distributed between the two genepools in order to maximally increase the heterosis in the F_1 hybrid.

5 Summary

Heterosis, or hybrid vigour, refers to the phenomenon that progeny of diverse inbred lines exhibit greater biomass, speed of development and fertility than the better of the two parents or the midparent value between them. While the practical application of heterosis in plant breeding is quite successful in many crops through the development of hybrid varieties, the basic understanding of the phenomenon is not very advanced. In the current study a doubled haploid population of 250 lines and their corresponding testcross hybrids were grown and analysed in greenhouse and field experiments at four locations in order to generate phenotypic data for studying heterosis in rapeseed on the QTL level. The main objectives included:

1. Identification of the levels of heterosis for agronomic important traits
2. Identification, localization and determination of the effects of QTL for heterotic traits (grain yield and yield components, early plant biomass, plant height), phenological traits (beginning of flowering, end of flowering and duration of flowering) and quality traits (oil, protein, glucosinolate, erucic acid and sinapine content).
3. Assessment of the contributions of different genetic effects, e.g. dominance, overdominance and epistasis to the expression of heterosis in rapeseed
4. Study of the correlation between molecular marker heterozygosity and hybrid performance
5. Identification of “hot spots” for QTL involved in heterosis

To elucidate the genetic basis of heterosis in rapeseed QTL analysis was performed with three separate datasets, each of which provided information for different genetic effects. As a prerequisite for the QTL mapping a new genetic linkage map was constructed based on 250 doubled haploid lines developed from microspores of an F_1 hybrid of a highly heterotic cross between the winter cultivar ‘Express’ and a resynthesized line, ‘R53’.The datasets used for QTL mapping included data from the doubled haploid population itself, a testcross hybrid population developed from crosses of the doubled haploid lines with the male sterile tester ‘MSL-Express’, and the midparent heterosis between the doubled haploid lines and their corresponding testcrosses. Using the three different datasets, the additive and dominance effects of the loci controlling the studied traits, as well as epistatic interactions contributing to trait variation could be estimated.

The phenotypic data used for QTL mapping was derived from a field trial carried out in one year at four locations following alpha lattice design of the type 26 x 10. The 250 doubled haploid lines and their corresponding testcross hybrids were grown in different beds on the

field and each doubled haploid line was followed by its corresponding testcross hybrid, which allowed the two genotypes to be grown in as similar conditions as possible, excluding the competition between the lines and the more vigorous hybrids.

Out of 14 traits studied, the highest levels of heterosis were observed for early fresh biomass and total grain yield, indicating that more complex traits show higher heterotis. Considering 9 heterotic traits (early fresh biomass, grain yield, thousand kernel weight, seeds per silique, siliques per square decimeter, plant height, beginning of flowering, end of flowering, and duration of flowering) 60 QTL with additive effects were identified using data of the doubled haploid population. Twenty seven of them were congruent with loci exhibiting dominance effects in the testcrosses, which allowed the assessment of the degree of dominance. Of the 27 QTL 11 showed partial dominance, 2 full dominance, and 14 exhibited overdominance. From 37 QTL mapped for 5 seed quality traits (oil, protein, glucosinolate, erucic acid, and sinapine content) showing very low or no heterosis only 3 QTL demonstrated overdominance, while from the remaining 8 QTL with dominance effects one showed full dominance and 7 exhibited partial dominance. QTL mapping results for erucic acid and glucosinolate content indicated that the lack of heterosis at population level does not exclude the existence of dominance at locus level. For these two traits loci were observed with positive and negative dominance effects, whose absolute values were of similar magnitude. Most probably the cancelling of the dominance effects with opposite signs led to the lack of heterosis at population level. The QTL mapping for loci involved in epistasis resulted in the localisation of a total number of 99, 112 and 101 epistatic locus pairs mapped with doubled haploid line, midparent heterosis, and testcross hybrid data, respectively. Most of the digenic interactions (85%) were between loci showing no significant main effects.

The results of the current study indicated that all levels of dominance in the range from partial dominance to overdominance play a role in the expression of heterosis in the rapeseed population under study. The large number of epistatic interactions observed showed that epistasis also contributes to heterosis in rapeseed, often explaining as large or a larger portion of the phenotypic variance than the main effect QTL.

In general the degree of correlation between genome heterozygosity and phenotypic traits reflects the importance of overall genome heterozygosity to trait expression. In the current study no correlation was observed between the overall genome heterozygosity and heterosis. The lack of correlation and the QTL mapping results demonstrated that there exist specific loci, often showing overdominance, which are responsible for the expression of heterosis,

meaning that the heterozygous status of these particular loci is much more important for the increased hybrid vigour than the overall genome heterozygosity.

Three regions in the rapeseed genome on linkage groups N11, N12, and N19, were identified as 'hot spots' for QTL as an apparent clustering of QTL with additive and dominance effects was observed at these sites. The three clusters included QTL for early fresh biomass, grain yield, thousand kernel weight, seeds per silique, siliques per square decimeter, plant height, beginning of flowering, end of flowering, duration of flowering, oil content, and glucosinolate content, mapped with at least one of the three datasets. Despite the extreme genetic complexity of the studied traits, with numerous epistatic interactions influencing heterosis, which would impede marker assisted selection (MAS), some major QTL with relatively large dominance effects and the three 'hot spots' for heterotic QTL could be of further interest for practical breeding. With the help of MAS the alleles of QTL showing overdominance could be purposefully distributed between complementary heterotic gene pools in order to benefit maximally the heterosis in F_1 hybrids derived from crosses between these pools.

By identifying QTL involved in heterosis the current study for the first time provided information on the contribution of different genetic effects, e.g. partial, full, overdominance, and epistasis to the expression of heterosis in rapeseed. QTL mapping in a population derived of a single cross is only the first step in the molecular dissection of a quantitative trait. A second step should include a verification of the current results in other populations. Further, fine mapping of selected QTL with strong effects on heterosis could be carried out in advanced backcross populations developed with the help of MAS.

6. Zusammenfassung

Der Begriff Heterosis oder Hybridwüchsigkeit bezieht sich auf das Phänomen, dass die direkten Nachkommen genetisch unterschiedlicher Inzuchtlinien oft eine größere Biomasse, schnellere Entwicklung und größere Fertilität aufweisen, als das Elternmittel oder sogar der bessere Elter. Während die praktische Anwendung der Heterosis in der Pflanzenzüchtung durch die Entwicklung von Hybridsorten bei vielen Kulturpflanzen sehr erfolgreich ist, ist das wissenschaftliche Verständnis des Phänomens nicht weit fortgeschritten. In der vorliegenden Studie wurden eine DH-Population von 250 Linien zusammen mit den korrespondierenden Testkreuzungen im Gewächshaus und in Feldversuchen an vier Orten angebaut und analysiert um phänotypische Daten für eine Analyse der Heterosis bei Raps auf der Ebene einzelner QTL zu gewinnen. Die wesentlichen Ziele der Studie waren dabei:

1. Bestimmung der Höhe der Heterosis für agronomisch wichtige Merkmale.
2. Identifizierung und Lokalisation sowie Schätzung der phänotypischen Effekte von QTL für heterotische Merkmale (Kornertrag und Ertragskomponenten, frühe Biomasse, Pflanzenhöhe), phenologische Merkmale (Blühbeginn, Blühende und Blühdauer) und Qualitätsmerkmale (Öl-, Protein-, Glucosinolat-, Erucasäure- und Sinapingehalt).
3. Bestimmung der Beiträge unterschiedlicher genetischer Effekte wie Dominanz, Überdominanz und Epistasie zur Ausprägung der Heterosis in Raps
4. Untersuchung der Korrelation zwischen Heterozygotie – bestimmt mit molekularen Markern – und der Hybridleistung
5. Nachweis von „hot spots“ für QTL, die die Heterosis kontrolieren

Um die genetische Basis der Heterosis in Raps aufzuklären wurden QTL-Analysen in drei verschiedenen Datensätzen durchgeführt, die es erlaubten, jeweils unterschiedliche genetische Effekte von QTL zu erfassen. Als Voraussetzung für die QTL-Kartierungen wurde zunächst eine neue genetische Kopplungskarte in einer spaltenden F_1DH-Population von 250 Linien aus einer Kreuzung zwischen der Winterrapssorte ‚Express’ und der resynthetisierten Linie ‚R53’ erstellt. Bei den für die QTL-Kartierung verwendeten Datensätzen handelte es sich um die phänotypischen Daten der DH-Population selbst, den Daten einer Population von Testhybriden aus Kreuzungen zwischen den DH-Linien und einer männlich sterilen Linie von ‚Express’ (MSL-Express) sowie der Heterosis der Testhybriden, jeweils bezogen auf das Elternmittel zwischen DH-Linie und zugehöriger Hybride. Mit Hilfe dieser Datensätze

konnten die Additiv- und Dominanzeffekte sowie epistatischen Interaktionen der Loci, welche die untersuchten Merkmale kontrollieren, geschätzt werden.

Die phänotypischen Daten für die QTL-Kartierung wurden in einjährigen Feldversuchen an vier Orten erhoben, die entsprechend einer Gitter Anlage des Typs 26 x10 aufgebaut waren. Um auf der einen Seite Konkurrenzeffekte zwischen den DH-Linien und den wüchsigeren Hybriden auszuschließen, die korrespondierenden Genotypen aber unter so ähnlichen Bedingungen wie möglich zu prüfen, wurden DH-Linien und Hybriden in getrennten aber parallelen Beeten angebaut, wobei DH-Linie und korrespondierende Hybride jeweils an gleicher Position im entsprechenden Beet standen.

Von den 14 untersuchten Merkmalen zeigten die frühe Biomasse und der Kornertrag die höchsten Heterosiswerte, was darauf hindeutet, dass komplexere Merkmale eine höhere Heterosis aufweisen. Für die neun untersuchten heterotischen Merkmale (frühe Biomasse, Kornertrag, Tausendkorngewicht, Samen pro Schote, Schoten pro dm^2, Pflanzenhöhe, Blühbeginn, -ende und –dauer) konnten 60 QTL mit additiven Effekten im Datensatz der DH-Population identifiziert werden. Von 27 dieser QTL, die kongruent mit Loci waren, die im Datensatz der Testkreuzungen bzw. im Heterosisdatensatz Dominanzeffekte gezeigt hatten, konnte der Dominanzgrad bestimmt werden. Dabei zeigten 11 QTL partielle Dominanz, 2 volle Dominanz und 14 Überdominanz. Von den 37 QTL, die für die fünf Qualitätsmerkmale (Öl-, Protein-, Glucosinolat-, Erucasäure- und Sinapingehalt) kartiert werden konnten, bei denen nur sehr geringe oder keine Heterosis beobachtet worden war, zeigten nur 3 QTL Überdominanz, einer volle und 7 partielle Dominanz. Die Ergebnisse der QTL-Kartierung für Erucasäure- und Glucosinolatgehalt zeigten andererseits, dass das Fehlen von Heterosis auf der Merkmalsebene nicht die Existenz von Dominanzeffekten an einzelnen Loci ausschließt. Bei beiden Merkmalen wurden QTL mit positiven wie negativen Dominanzeffekten beobachtet, die sich, da von vergleichbarer Größenordnung, in der Population aufheben. Die Analyse epistatischer Interaktionen in den Datensätzen der DH-Population, der Heterosis und der Testkreuzungen führte zu Kartierung von 99, 112 bzw. 101 Locuspaaren mit signifikanten digenen Interaktionen. Die meisten dieser Interaktionen (85) traten zwischen Loci auf, die als Einzelloci keinen signifikanten Effekt gezeigt hatten.

In der gegenwärtigen Studie wurde bei keinem Merkmal eine signifikante Korrelation zwischen der allgemeinen, genomweiten Heterozygotie und der Höhe der Heterosis gefunden. Dieses Ergebnis und die Ergebnisse der QTL-Kartierungen zeigen deutlich, dass die Heterosis beim Raps durch spezifische Loci, die häufig Überdominanz zeigen, bestimmt wird, d. h. der

heterozygote Zustand an diesen spezifischen Loci ist weit wichtiger für die Hybridwüchsigkeit als die mittlere Heterozygotie des Genotyps.

In drei Regionen des Rapsgenoms auf den Kopplungsgruppen N11, N12 und N19 konnten sog. „hot spots“ für QTL identifiziert werden, die eine auffällige Häufung von QTL mit Additiv- und Dominanzeffekten enthielten. Die drei Regionen enthalten QTL für frühe Biomasse, Kornertrag, Tausendkorngewicht, Samen pro Schote, Schoten pro dm^2, Pflanzenhöhe, Blühbeginn, -ende und –dauer sowie Öl- und Glucosinolatgehalt. Trotz der extremen genetischen Komplexität der untersuchten Merkmale bei denen die Heterosis und Merkmalsausprägung durch eine Vielzahl von epistatischen Interaktionen beeinflusst wird, was eine markergestützte Selektion erschweren würde, könnten einige QTL mit relativ großen Dominanzeffekten und die drei „hot spots“ für die praktische Züchtung von Interesse sein. Mit Hilfe der markergestützten Selektion könnten die Allele von QTL, die Überdominanz gezeigt haben, gezielt auf unterschiedliche Genpools verteilt werden, um die Heterosis in F_1-Hybriden aus Kreuzungen zwischen den Pools zu maximieren.

Durch die Kartierung von QTL, die an der Ausprägung von Heterosis beteiligt sind, hat die vorliegende Studie das erste Mal Erkenntnisse zum Beitrag verschiedener genetischer Effekte, also partielle, volle und Überdomianz sowie epistatische Interaktionen, zur Heterosis im Raps geliefert. Die QTL-Kartierung in einer Population die von einer einzelnen Kreuzung stammt kann aber nur der erste Schritt in einer molekularen Analyse eines quantitativen Merkmals sein. Ein zweiter Schritt sollte die Verifikation der gegenwärtigen Ergebnisse in anderen Populationen einschließen. Weiterhin könnte eine Feinkartierung ausgewählter QTL mit starken Effekten auf die Heterosis in fortgeschrittenen Rückkreuzungspopulationen, die über markergestützte Selektion zu entwickeln wären, durchgeführt werden.

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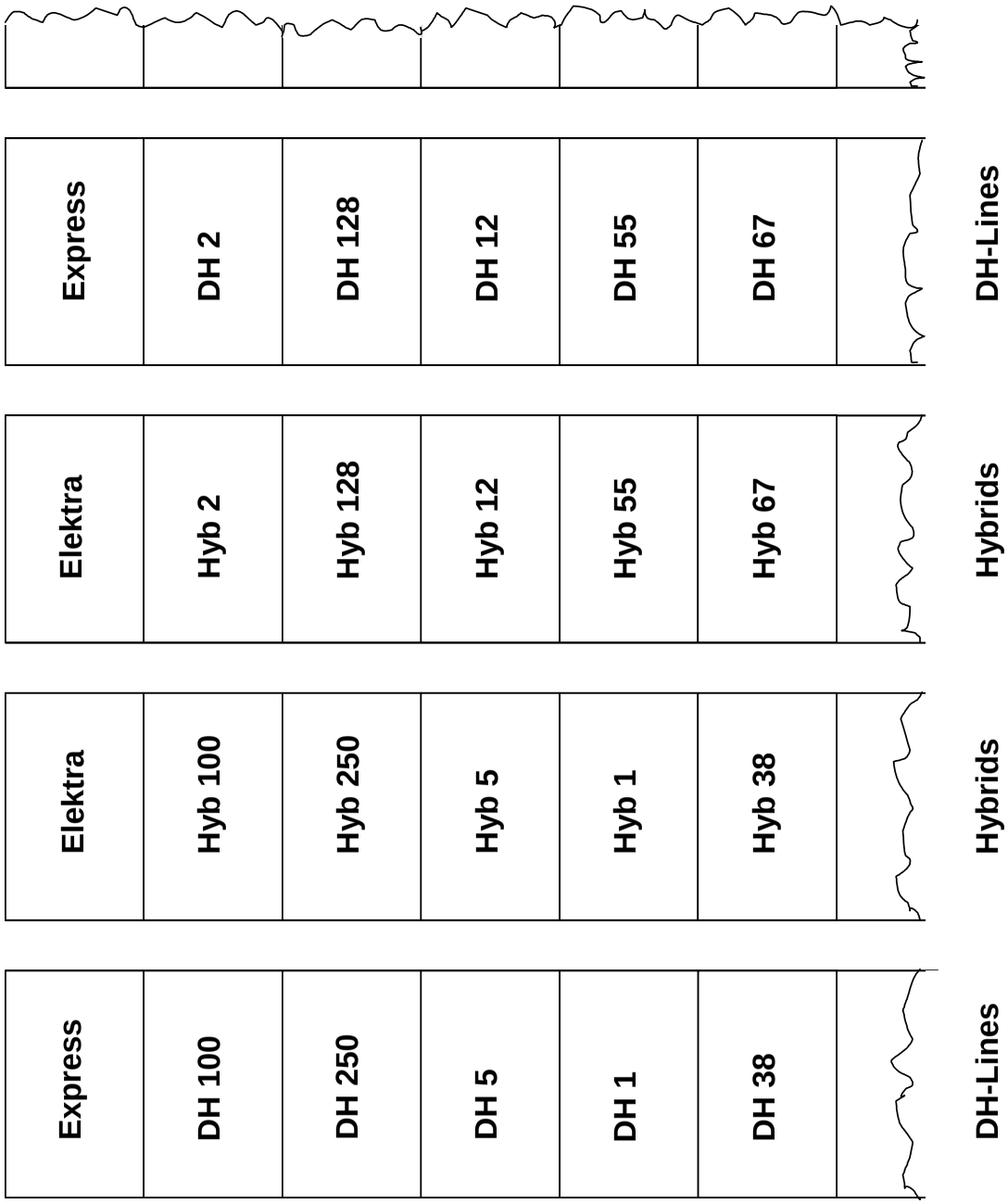
Appendices

Appendix 1 List of the laboratory chemicals used

Chemicals	Producers	Branch offices
Nucleon®PhytoPure®	Amersham Biosciences	Germany, Freiburg
Bio-Rad Fluorescent DNA Quantification Kit	Bio-Rad Laboratories	Germany, Munich
EcoRI	MBI Fermentas	Germany, St. Leon-Rot
MseI	New England Biolabs	Germany, Frankfurt am Main
FIREPol®Taq-polymerase	Solis Bodyne	Estonia, Tartu
T4 DNA ligase	Promega	Germany, Mannheim
ATP	Sigma-Aldrich Chemie	Germany, Deisenhofen
dNTPs	Qbiogene	Germany, Heidelberg
Hi-Di™Formamide	Applied Biosystems	Germany, Darmstadt
GeneScan™-500 ROX™	Applied Biosystems	Germany, Darmstadt
POP6 Polimer	Applied Biosystems	Germany, Darmstadt
Tris	MP Biochemicals	Germany, Eschwege
EDTA	ROTH	Germany, Karlsruhe
Isopropanol	ROTH	Germany, Karlsruhe
Chlorophorm	ROTH	Germany, Karlsruhe
Mercapthoetanol	Merck-Schuchardt	Germany, Hochenbrunn
HPLC H_2O	J. T. Baker	The Netherlands, Deventer

Appendix 2 Schematic representation of the field design**.** The doubled haploid lines and the hybrids were grown in different beds on the field. Each doubled haploid line was followed by its corresponding hybrid.



Apendix 3 Derivation of the genetic effects, using different datasets for QTL mapping, in all cases the cultivar 'Express' was used to express the performance of the different genotypes.

i) Main effects

1. Doubled haploid population

$$effect = \frac{EE - RR}{2}$$

- 'Express' contributes the increasing allele (Fig. 1 A)

$$effect = \frac{EE - (EE - 2a)}{2} => effect = a$$

- 'R53' contributes the increasing allele (Fig. 1 B)

$$effect = \frac{EE - (EE + 2a)}{2} => effect = -a$$

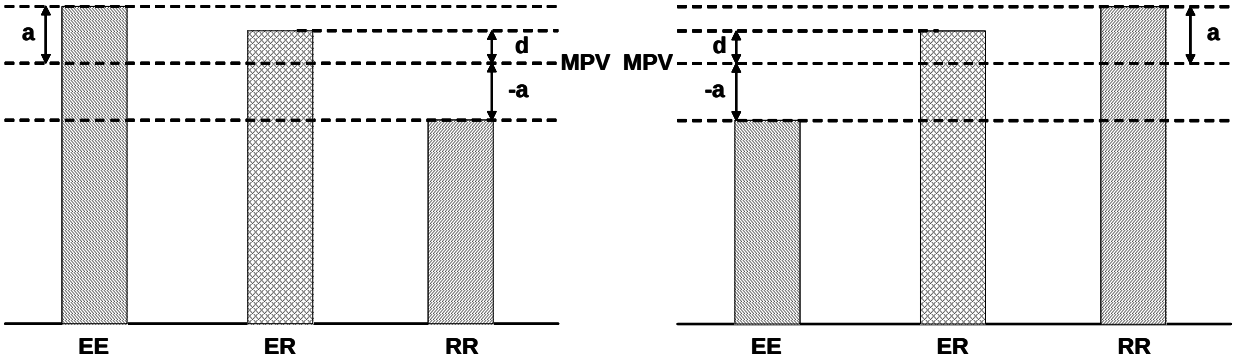


Fig. 1 Graphical presentation of the metric effects, E and R designate alleles of 'Express' and 'R53' respectively. 'a' and 'd' are additive and dominance effects. In A 'Express' contributes the increasing allele. In B the increasing allele is contributed by 'R53', the presented dominance effect is partial dominance.

2. Testcross hybrid population

$$effect = \frac{EE - ER}{2}$$

- 'Express' contributes the increasing allele (Fig. 1 A)

$$effect = \frac{EE - (EE - a + d)}{2} => effect = \frac{a - d}{2}$$

- 'R53' contributes the increasing allele (Fig. 1 B)

$$effect = \frac{EE - (EE + a + d)}{2} => effect = -\frac{a + d}{2}$$

3. Midparent heterosis data

$$effect = \frac{Dev_1 - Dev_2}{2}$$

- 'Express' contributes the increasing allele (Fig. 1 A)

$$Dev_1 = EE - MPV, MPV = \frac{EE + EE}{2}$$

$$Dev_1 = EE - \frac{EE + EE}{2} = 0$$

$$Dev_2 = ER - MPV, MPV = \frac{EE + RR}{2}$$

$$Dev_2 = EE - a + d - \frac{EE + EE - 2a}{2} = d$$

$$effect = \frac{0 - d}{2} = -\frac{d}{2}$$

- 'R53' contributes the increasing allele (Fig. 1 B)

$$Dev_1 = EE - MPV, MPV = \frac{EE + EE}{2}$$

$$Dev_1 = EE - \frac{EE + EE}{2} = 0$$

$$Dev_2 = ER - MPV, MPV = \frac{EE + RR}{2}$$

$$Dev_2 = EE + a + d - \frac{EE + EE + 2a}{2} = d$$

$$effect = \frac{0 - d}{2} = -\frac{d}{2}$$

ii) Epistatic interactions

A total number of 9 hypothetical genotypes is possible, if two gene loci are considered. These genotypes can be described by a quantitative genetic model, which includes 8 parameters: two additive effects a_A and a_B, two dominance effects d_A and d_B, and four epistatic effects aa_{AB}, ad_{AB}, ad_{BA}, and dd_{AB} (Table 1).

Table 1 Digenic epistasis model with 8 parameters, F_∞-metric notation follows Kearsey and Pooni (1996)

	B^+B^+	B^+B^-	B^-B^-
A^+A^+	$a_A+a_B+aa_{AB}$	$a_A+d_B+ad_{AB}$	$a_A-a_B-aa_{AB}$
A^+A^-	$a_B+d_A+ad_{BA}$	$d_A+d_B+dd_{AB}$	$-a_B+d_A-ad_{BA}$
A^-A^-	$-a_A+a_B-aa_{AB}$	$-a_A+d_B-ad_{AB}$	$-a_A-a_B+aa_{AB}$

1. Doubled haploid population

   Possible allele combinations at loci A and B in a doubled haploid population. *E*, represents an allele coming from the parent 'Express', *R* is an allele contributed by 'R53'.

A	**B**
EE	*EE*
EE	*RR*
RR	*EE*
RR	*RR*

   $effect = (EEEE + RRRR) - (EERR + RREE)$
   $effect = (a_A + a_B + aa_{AB} - a_A - a_B + aa_{AB}) - (a_A - a_B - aa_{AB} - a_A + a_B - aa_{AB})$
   $effect = 2aa_{AB} + 2aa_{AB} = 4aa_{AB}$

2. Testcross hybrid population

   Possible allele combinations at loci A and B in a testcross population (tester genotype *EEEE*). *E*, represents an allele coming from the parent 'Express', *R* is an allele contributed by 'R53'.

A	**B**
EE	*EE*
EE	*ER*
ER	*EE*
ER	*ER*

   $effect = (EEEE + ERER) - (EEER + EREE)$
   $effect = (a_A + a_B + aa_{AB} + d_A + d_B + dd_{AB}) - (a_A + d_B + ad_{AB} + d_A + a_B + ad_{BA})$
   $effect = aa_{AB} + dd_{AB} - ad_{AB} - ad_{BA}$

3. Midparent heterosis data

   Possible allele combinations at loci A and B in a testcross population (tester genotype *EEEE*), and the corresponding midparent values. *E*, represents an allele coming from the parent 'Express', *R* is an allele contributed by 'R53'.

TC			**MPV**	
A	**B**		**A**	**B**
EE	*EE*	-	*EE*	*EE*
EE	*ER*	-	*EE*	*(EE+RR)/2*
ER	*EE*	-	*(EE+RR)/2*	*EE*
ER	*ER*	-	*(EE+RR)/2*	*(EE+RR)/2*

   Midparent values:

$$EEEE = \frac{EEEE + EEEE}{2} = a_A + a_B + aa_{AB}$$

$$EE\,\frac{EE + RR}{2} = \frac{EEEE + EERR}{2} = \frac{a_A + a_B + aa_{AB} + a_A - a_B - aa_{AB}}{2} = a_A$$

$$\frac{EE+RR}{2}EE=\frac{EEEE+RREE}{2}=\frac{a_A+a_B+aa_{AB}-a_A+a_B-aa_{AB}}{2}=a_B$$
$$\frac{EEEE+RRRR}{2}=\frac{a_A+a_B+aa_{AB}-a_A-a_B+aa_{AB}}{2}=aa_{AB}$$

Genetic effect:

$$effect=EEEE-EEEE+ERER-\frac{EEEE+RRRR}{2}-\left(EEER-EE\frac{EE+RR}{2}+EREE-\frac{EE+RR}{2}EE\right)$$
$$=0+d_A+d_B+dd_{AB}-aa_{AB}-\left(a_A+d_B+ad_{AB}-a_A+d_A+a_B+ad_{BA}-a_B\right)$$
$$=dd_{AB}-aa_{AB}-ad_{AB}-ad_{BA}$$

Appendix 4 *EcoRI* and *Mse*I primers with one and three selective nucleotides.

a) *EcoRI* and *Mse*I primers with one selective nucleotide

E01	5’-CTGCGTACCAATTCA-3’
M02	5’-GATGAGTCCTGAGTAAC-3’

b) *EcoRI* and *Mse*I primers with three selective nucleotides

E32	5’-CTGCGTACCAATTCAAC-3’
E33	5’-CTGCGTACCAATTCAAG-3’
E35	5’-CTGCGTACCAATTCACA-3’
E38	5’-CTGCGTACCAATTCACT-3’
E40	5’-CTGCGTACCAATTCAGC-3’
M47	5’-GATGAGTCCTGAGTAACAA-3’
M48	5’-GATGAGTCCTGAGTAACAC-3’
M49	5’-GATGAGTCCTGAGTAACAG-3’
M50	5’-GATGAGTCCTGAGTAACAT-3’
M51	5’-GATGAGTCCTGAGTAACCA-3’
M59	5’-GATGAGTCCTGAGTAACTA-3’
M60	5’-GATGAGTCCTGAGTAACTC-3’
M61	5’-GATGAGTCCTGAGTAACTG-3’
M62	5’-GATGAGTCCTGAGTAACTT-3’

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